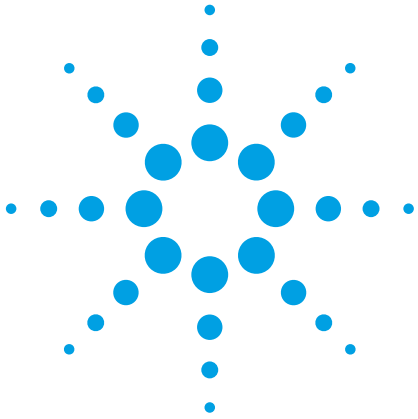
Agilent 3396
Series III Integrator

Operating Manual

Agilent Technologies

Agilent 3395/3396 Integrators



Manuals

These manuals may contain references to HP or Hewlett-Packard. Please note that Hewlett-Packard's former test and measurement, semiconductor products and chemicals analysis businesses are now part of Agilent Technologies. The HP 3395/3396 Integrator referred to throughout this document is now the Agilent 3395/3396 Integrator.

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Safety Information

The HP 3396 Series III meets the following IEC (International Electrotechnical Commission) classifications: Safety Class 1, Transient Overvoltage Category II, and Pollution Degree 2.

This unit has been designed and tested in accordance with recognized safety standards and designed for use indoors. If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be impaired.

Whenever the safety protection of the HP 3396 Series III has been compromised, disconnect the unit from all power sources and secure the unit against unintended operation.

The manual reference sign is marked on the product when it is necessary for the user to refer to the instruction manual. If the procedure or practice described in the manual is not followed, loss of data or damage to the instrument could result.

WARNING A warning calls attention to a condition or possible situation that could cause you or others injury.

Caution A caution calls attention to a condition or possible situation that could damage or destroy the product or your work.

Important User Information for In Vitro Diagnostic Applications

This is a multipurpose product that may be used for qualitative or quantitative analyses in many applications. If used in conjunction with proven procedures (methodology) by a qualified operator, one of these applications may be In Vitro Diagnostic Procedures.

General instrument performance characteristics and instructions are included in this manual. Specific In Vitro Diagnostic procedures and methodology remain the choice and the responsibility of the user and are not included.

Manual Addendum

This manual addendum addresses changes to sequence operation in remote start/stop (RSS) configurations of the HP 3396 Series III integrator. The operation of the system in RSS mode is explained in more detail than is found in the current manual. Usually a non-HP automatic liquid sampler (ALS) will be part of your system when you operate in RSS mode, so you may need to obtain additional information from your ALS manufacturer.

Sequence Operation in RSS Configuration

A configuration in which an RSS cable supplies the communication between the ALS and the HP 3396 Series III integrator is an RSS configuration. In this configuration, the sequence content and injection parameters are controlled by the ALS. The integrator can receive bottle number information for the ALS if a bottle number cable is installed or can identify the sequence by run number.

If you are an experienced HP 3395 Series II integrator user, you will notice improvements in the sequence capabilities of the HP 3395 Series III integrator when operating with non-INET samplers and a new NEXT SEQUENCE function that allows sequences to be chained within the sequence preparation dialog. To take advantage of these improvements, you must operate the sequence differently than with previous HP 3396 integrators.

You can still allow the sequence to be controlled completely by the ALS. In this mode of operation, the HP 3396 Series III integrator sees the sequence as a series of manual runs. This is how the HP 3396 Series II integrator operated. If you chose to operate in this mode, follow the instructions in the *HP 3396 Series III Integrator Operating Manual*, pages 9-19 to 9-25. The disadvantage of using the integrator in this manner is that the new integrator features designed to allow better management of RSS operation cannot be used. For example, the NEXT SEQUENCE function will not be available (because you are really doing manual runs).

The HP 3396 Series III integrator must participate in the control of an RSS sequence to utilize the new sequencing features. To operate in this mode, prepare the sequence by following the instructions on pages 9-19 to 9-25 in the *HP 3396 Series III Integrator Operating Manual*, but skip the [OP()][7] dialog.

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Meet the HP 3396 Series III

Meet the HP 3396 Series III

The New HP 3396

This manual set is for the HP 3396 Series III. The two major enhancements in the HP 3396 Series III are that it is designed to take full advantage of the programming capabilities of the HP 6890 GC, and it has greatly increased data storage capacity.

The internal memory, or M: disk, of the HP 3396 Series III has a capacity of 256 Kbytes. It can be configured to an external disk drive or host computer; in addition, the HP 3396 Series III has two slots in the back panel that will accept expansion cards. See Chapter 7 for a detailed explanation of expansion cards.

There are several improvements in the sequence capabilities of the HP 3396 Series III. Operation with non-INET samplers has been improved, and a new NEXT SEQUENCE function allows sequences to be chained within the sequence preparation dialog. The HP 3396 Series III operates differently with the HP 6890 than with earlier GC models. See Chapter 9, *Automating Analyses*, for more information about sequences.

The HP 3396 is available as a general purpose instrument, or with Application Programs designed for either liquid chromatography or gas chromatography use. The generic instrument is designated as the HP 3396 Series III on the keyboard label. It contains general application programs, which are explained in detail in the *Using Application Programs* manual. The keyboard overlay that identifies the general applications programs says “HP Integrator Application Programs/General.”

The HP 3396 Dual Channel integrator contains two additional application programs of most use for gas chromatography applications. The HP 1050 LC Control Applications Package contains the generic programs plus an “LC START” program. A general purpose integrator can be upgraded to contain the additional application programs.

If you have purchased your HP 3396 as part of a package with the HP 6890 Gas Chromatograph, the keyboard label will say “HP 6890 SERIES Integrator.” This integrator contains the Dual Channel Applications Programs in addition to the generic programs.

An integrator with the Headspace Control Applications Program contains the generic programs plus a program that controls the Headspace Module.

Using the Keyboard

HP 3396 SERIES III INTEGRATOR
KEYBD PREP EDIT STORE LOAD SEQ METH CALIB REPORT AREA% OP( ) COMM READY RUN
LIST DEL ZERO ATT STOP START

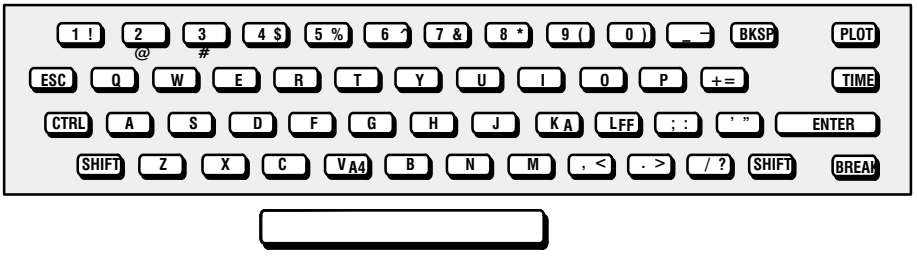
The top row of the HP 3396 keyboard is a group of dedicated keys for the most frequently used functions. Each key has two values; reach the second one by holding the **[SHIFT]** key as on a typewriter or PC keyboard. Functional keys are shown in brackets [ ] with **boldface** type. **[LIST]** is the first key in the top row; **[PREP]** is its shifted form.

Example **[LIST] [METH] [ENTER]**

```
*  LIST: METH @
```

In this example the method key is reached by holding down [**SHIFT**] and pressing the [**AR REJ**] key.

A complete list of all the functional keys is located later in this chapter.



The QWERTY-like alphanumeric keyboard is used for the rest of the commands and parameter values. Number or letter keys appear in this book as **[1]**, **[2]**, **[A]**, **[B]** and so forth. Multiple digit numbers such as 123 appear as **[1] [2] [3].**

System commands are typed and shown in **boldface** type, *without* brackets. A complete list of all system commands is located later in this chapter.

Example **HELP [ENTER]**

```
*HELP

ANALYZE  ASSIGN  BASIC  BX  COPY  CREATE  DATE  etc
```

The integrator accepts a command following a prompt. The three possible prompts are:

- `*` Ready to accept system level commands.
- `>` Ready to accept BASIC commands and program statements. Enter from `*` by typing **BA**. Return to `*` by typing **EXIT**.
- `#` Instrument is being controlled by a host computer. See the HP 3396 Reference Manual or the HP Peak-96 Information Manager User's Guide.

Conventions

Optional parts of the commands are enclosed in { }. Values appear as *value description* (in italics).

Example Thirty seconds into the run, set the chart speed to 7.

```
*  TIME  .5   CHT SP   7   @
```

is entered by pressing these keys in this order:

[TIME]	on the right side of the keyboard
runtime	a numeric value, in minutes after injection
key	a function key, which is to execute at *runtime*
{*value*}	a numeric value, if *key* requires one
[ENTER]	indicates the end of *value* and terminates the command

Commands execute as soon as all the needed information is entered. In the example above, the **[ENTER]** key (a delimiter) is needed because there is no other way to know when all the digits for *value* have been entered.

A command without parameters, however, such as **[LIST] [CHT SP]**, executes as soon as the second key is pressed.

Example `* LIST: CHT SP = 1.0`

A few key combinations require that the **[CTRL]** key be held while typing another key. The effect is similar to the **[SHIFT]** key; it changes the default value of the key in question.

Example

1. **Press [CTRL] and hold it while you press [L] to form feed the printer paper.**

Instructional steps (like the one shown in the example above) are listed in **boldface**. Supporting information is always indented below the step.

Correcting Mistakes

1. **Press [BKSP] if you notice a typing error before pressing a delimiter key such as [ENTER].**

   The printer doesn't back up as a typewriter does. A block character is printed instead, and the character to the left of the block is erased from the integrator's memory. It's still on the paper, but gone from memory.

   You also have the option to press **[ESC]** to cancel the entire entry and start again.

2. **Type the correct character.**
3. **Press [CTRL] and hold it while you press [R] to reprint the corrected line.**

Example **[D] [A] [T] [R] [BKSP] [E] [4] [/] [1] [4] [/] [9] [0] [CTRL-R]** shows up on the printer as

```
*  DATR E 4/14/95 =
DATE 4/14/95
```

Error Indications and What to Do	
Beep	A command is ignored when entered during plotting, integrating, or reintegrating. Either wait or end the operation and try again. Can also happen if you type faster than the HP 3396 can accept input.
?	A numeric entry is out of limits. Enter a correct value after the ? or press **[ESC]** and try again.
`INVALID SYSTEM COMMAND`	This command doesn't exist. Usually a typing error; enter the command again after the ? or press **[ESC]** and try again.

Pressing **[BREAK]** will cancel the entire command and return to `*`

Pressing **[CTRL] [BREAK]** restarts the instrument, similar to the power-on routine. All setpoints and data on the memory disk are saved. However, any BASIC program in the BASIC workspace will be lost.

Using the Function Keys

Function Key	What the Key Does	See Chapter...
AR REJ	Sets the minimum areas for peaks to be reported.	3
AREA%	Produces an uncalibrated report using active workspace data.	6
ATT 2↑	Sets the scale of the signal axis for plotting.	2
CALIB	Performs operations on calibrations.	5
CHT SP	Sets the paper advance rate for the plotter.	2
DEL	Deletes all or part of a calibration, method, or sequence.	2,5,8,9
EDIT	Modifies part of a calibration, method, or sequence.	5,8,9
EXT( )	Accesses External Device Controls. See table on next page.	9
INTG( )	Accesses Integration functions. See table on next page.	3,4
L FF	With CTRL key formfeeds paper to next top of form.	2
LIST	With other function keys, prints value of function. Alone, prints run parameters.	2,5,8,9
LOAD	Copies a calibration, method, or sequence file into the active workspace from memory.	5,8,9
METH	Performs operations on methods.	8
OP( )	Accesses options dialogs. See table later in this chapter.	1
PK WD	Sets the expected width for peaks to optimize quantitation.	3,4
PLOT	Plots the signal. (No data integration takes place)	2
PREP	Starts the preparation dialog for calibrations, methods, or sequences.	5,8,9
REPOR	Produces a report using data in the active workspace and specified calibration type.	10
SEQ	Performs operations on sequences.	9
START	Begins an integration run.	2,3
STOP	Terminates integration or plotting.	2,3
STORE	Saves a calibration, method, or sequence.	5,8,9
THRSH	Sets the minimum height for peak detection.	3,4
TIME	Used with LIST and DEL to perform operations on the time table.	2,9
V A4 K A	With CTRL key sets form size.	2
ZERO	Sets the position of the chromatographic baseline on the chart.	2

Using the Multifunction Keys

External Device Control Key [EXT()]

The External Device Control key controls a set of eight 250 V relay switches in an HP 19405B INTEG ECM. This key is discussed in detail in chapter 9, *Automating Analyses*.

1. **Press [LIST] [EXT()] [ENTER] for a complete listing of the external events.**

Integration Function Key [INTG()]

The Integration Functions alter the default actions of the integration software. Chapter 3 discusses the use of these keys; chapter 4 provides a detailed description of each one.

1. **Press [LIST] [INTG()] [ENTER] for a complete listing of the integration functions.**

Option Keys [OP()]

The option keys allow you to specify various method parameters, access remote INET devices, or enter sample information.

Number	Function	See...
OP() 1	**Integration Plot Type:** Select Source, Filtered, Unigram, or No Plot	Chp 2
OP() 2	**Run Data Storage:** Specify storage or nonstorage of signal data, storage device, bunched or raw data, and storage of processed peaks.	Chp 7
OP() 3	**Calibration Options:** Specify response factor for uncalibrated peaks, replacement of calibration fit, retention time updating, internal standard peak number, internal standard amount, sample amount, and multiplication factor.	Chp 5
OP() 4	**Report Options:** Specify local report suppression, HEIGHT% for uncalibrated reports, replacement of report title, replacement of amount label, reporting of uncalibrated peaks, and extended report format.	Chp 10
OP() 5	**Post-Run List Options:** Specify report storage and storage device, external postrun reporting, postrun listings of run parameters, timetable, calibration table, and the remote method, formfeed before and after report printing, and perforation skipping in reports and plots.	Chp 2 Chp 10
OP() 6	**Remote Device Access:** Send command strings to INET instruments or a host computer.	*
OP() 7	**Default Sample Information:** Specify default values for internal standard amount, sample amount, multiplication factor, name, and report memo.	Chp 9
OP() 8	**Range Setpoints:** Specify the data bit range of the HP 6890 (HP 3396 Series III only).	Chp 8

* See the HP 3396 Reference Manual.

1. **Press [LIST] [OP()] [ENTER] for a complete listing of the option keys.**

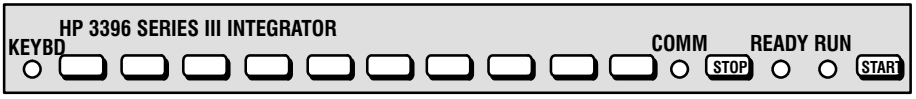
Using the System Commands

These commands are typed in on the alphanumeric keyboard; they do not have function keys. You need only type as many characters as are required to distinguish the desired command from others. For example, the **COPY** command may be entered as **CO**, **COP**, or **COPY** but not as **C**, since this is also the beginning of the **CREATE** command.

Command	Function	See Chapter...
ANALYZE	Start reintegration of stored run data.	**3**
ASSIGN	Assign a BASIC program to a numeric key for auto execution when the key is pressed.	**BASIC Language manuals**
BASIC	With BASIC installed. Enter BASIC mode.	
BX	With BASIC installed. Enter BASIC from a remote terminal.	
CREATE	Create a new file.	
COPY	Copy a file.	**7**
DATE	List or set the date.	**2**
DIRECTORY	List files and space on a storage device.	**7**
FORMAT	Prepare a disk for data transfer, with files using the HP 3396 Series II formats.	**7**
HELP	List all system commands.	**1**
IDENTIFIER	Enter a 12-character identifier for a report.	**6**
INET_CONFIG	List or modify the INET configuration.	**Reference Manual**
LOCK	Prevent communication from a host computer.	
NOTEPAD	Enter notations for the printer/plotter chart.	**10**
PACK	Recover fragmented space on a disk.	**7**
PURGE	Delete a file.	**7**
READY	Report on INET system readiness.	**9**
RECONFIGURE	Reconfigure the HP-IL/INET.	Reference Manual
RENAME	Change the name of a file.	**7**
SET	Set run parameters and the run number.	**9**
SSET ANALOG	Sets the analog reference signal to 0 or 1.	Reference Manual
SSET CARD_FIRST	Integrator assigns expansion card disk volumes first.	**7**
SSET HPIL_FIRST	Integrator assigns floppy disks first.	**7**
SSET RS232	Override RS232 default settings.	Reference Manual
SSET VOL	Sets the number of disk volumes on an expansion card.	**7**
SYSTEM	List HP-IL , INET, and RS-232C configurations— device numbers, instruments, or devices, settings.	**7**
TIME	List and set the time of day.	**2**
UNLOCK	Permit communication by a host computer.	**Reference Manual**
XADDRESS	Set the external address for CTRL LIST.	

Reading the Status Indicators

Four status indicators in the top row of keys show the present condition of the instrument.



Indicator	On	Blinking	Off
KEYBD	Keyboard command entry allowed.	No commands accepted; BX active or OUT OF PAPER.	No commands accepted; processing or listing data.
COMM	Host computer is in control of integrator	Integrator is in control and transmitting data.	No communi-cation activity.
READY	Integrator or INET is ready.	Auto-THRSH measurements in progress.	Integrator or INET not ready for start.
RUN	Run in progress.	Postrun operations in progress; can-not start run.	Ready for run to start.

Using Uppercase and Lowercase

By default, or after a long power failure, the HP 3396 prints all letters in uppercase (capitals). In this mode, you can press **[SHIFT]** to type lowercase (small) letters. You can change the keyboard mode to conventional typewriter style.

1. **Press [CTRL] and hold it while you press [C] to change the keyboard mode to conventional typewriter style (lowercase normally, [SHIFT] for uppercase).**

   This applies only to the letter keys. Regardless of which style is in use, all other two-valued keys give the value on the key normally and the value above the key if **[SHIFT]** is used.

2. **Press [CTRL] again and hold it while you press [C] to change the keyboard mode back to the original style.**

Using the Small Font

It is possible to use a smaller font than the one displayed when the instrument is turned on. This smaller font can be specified from the keyboard or through the option 5 dialog.

1. **Press [CTRL] and hold while pressing [D] to change to the small font.**

Example

```
*  REPORT

RUN#     65           OCT  5, 1994  09:01:32
                             SAMPLE#    7

SIGNAL FILE: M:SIGNAL.BNC

TECH PROPIONIC ACID
                the report continues...


[CTRL – D]
*  REPORT

RUN#     65           OCT  5, 1994  09:01:32
                             SAMPLE#    7

SIGNAL FILE: M:SIGNAL.BNC

TECH PROPIONIC ACID
                the report continues...
```

2. **Press [OP ()] [5] [ENTER] to specify use of the smaller font for a method.**

Example

```
*  OP # 5
PRINT & POST-RUN LIST OPTIONS
Large font  [Y*/N]:
```

3. **Press [N] [ENTER] to specify the smaller font.**

   All printing associated with this method will be in the smaller font.

Cold, Warm and Cool Starts

There are three types of power-up states for the HP 3396C. The integrator makes a *cold start* power-up whenever:

- it is first turned on;
- a power failure occurs for longer than the life of the back-up battery, which is about 95 hours;
- the [CNTRL] [DEL] keys are pressed simultaneously.

On a cold start, all of the data in the integrator memory (the M: disk) is lost, any program in the BASIC workspace is deleted, and all run parameters are set to their default values. After a cold start, the integrator performs a series of self tests and then brings the INET loop up.

A *warm start* power-up occurs when:

- there is a power failure shorter than 95 hours;
- the electrical cord is unplugged from the wall outlet or the back of the HP 3396, then plugged back in;
- the integrator is turned off, then on.

On a warm start, the files in memory and all run parameters are preserved. Any dialog or chromatographic run in progress will be aborted. The integrator prints a message indicating that there was a power failure before bringing the INET loop up.

An intermediate *cool start* state occurs when the integrator is reset with the [CNTRL] [BREAK] keys. In this state, any program that is present in the BASIC workspace is lost, but all other parameters are retained.

2

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Getting Started

Getting Started

Setting Date and Time

The HP 3396 has a calendar and a clock to date reports and "time stamp" files.

1. **Type [D] [A] [T] [E]** *mm/dd/yy* **[ENTER] to set the calendar.**

   mm/dd/yy is the month (mm = 01 to 12), the day (dd = 01 to 31), and the last 2 digits of the year (yy). The slash (/), colon (**:**), and comma (**,**) are all acceptable separators.

Example

```
* DATE 3/28/95
MAR 28, 1995 00:00:20
```

2. **Type [T] [I] [M] [E]** *hh***:***mm***:***ss* **[ENTER] to set the clock.**

   *hh***:***mm***:***ss* is the hour (hh = 00 to 23), minute (mm = 00 to 59), and seconds (ss = 00 to 59).

Note: The **[TIME]** key is not used to set the clock.

Example

```
* TIME 08:35:30
MAR 28, 1995   08:35:30
```

Example **[D] [A] [T] [E] [ENTER]** prints the current date and time.
[T] [I] [M] [E] [ENTER] prints the time only.

DATE and **TIME** can be abbreviated as **DA** and **T** respectively, since these are enough letters to distinguish them from all other system commands.

Starting the Plot or Integration

The HP 3396 operates as a simple signal plotter or as an integrator with a built-in plotter.

1. **Type [R] [E] [A] [D] [Y] [ENTER] to check system readiness.**
2. **Select integration plot type and chart control parameters or use default settings.**

   Instructions for setting these parameters are located later in this chapter.

3. **Press [PLOT] to begin plotting the signal.**

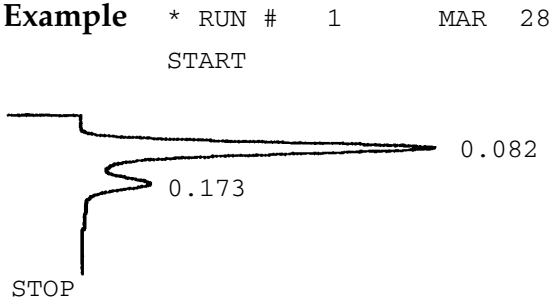
   In the plot-only mode, the integrator plots the current GC signal. If a run is started on the GC, the integrator draws the chromatogram but does not perform peak integration, calculation, or reporting. Such plots lack the printed retention times and event markers that appear during an integration run. **[PLOT]** does not start the GC.

Example `* PLOT`

`STOP`

4. **Press [START] to begin an integration (with plotting) run.**

Example



5. **Press [STOP] to end the run when peaks of interest have appeared on the plot.**

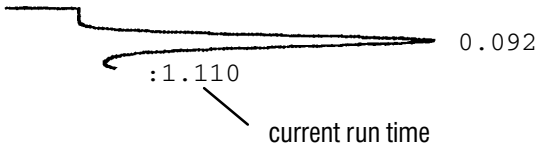
   An integration run can also be ended with a time-programmed **STOP**.

   See chapter 3 and *Understanding Time Programming* at the end of this chapter for related information.

Printing Run Time

1. **Press the [TIME] key during a run to print the current run time on the plot.**

Example `START`



Selecting Integration Plot Type

The plot type applies only to integrated or reintegrated runs.

1. **Press [OP()] [1] [ENTER] to select the plot type.**

Example

```
INTEGRATION PLOT TYPE
(Source/Filtered/Unigram/No Plot)
ENTER PLOT TYPE [S/F*/U/N]:
```

An * marks the current selection. F* (filtered) is the default plot format. Descriptions of plot choices are listed below.

2. **Press [ENTER] for a filtered plot. For another plot choice, type the appropriate letter, then press [ENTER].**

`Source Plot`

Plots the data being received by the instrument, whether analog or from disk. There is no noise suppression, filtering, or other signal "cleanup". Tick marks (start and stop of peak, for example) are not printed.

`Filtered Plot`

Default format that displays the data as seen by the integration software after noise suppression and filtering have occurred. Retention times are printed. Turning on Integration function 8 provides tick mark annotation. See chapter 6 for a description of tick marks.

`Unigram Plot`

Alters both the time and height axes of the plot. The effect spreads peaks evenly over a nonlinear time axis and makes peak heights on the plot proportional to peak areas. A unigram plot is useful in selecting values for the PK WD integration parameter. See *Optimizing Peak Recognition* in chapter 4 for related information on unigrams.

```
No Plot
```

No plot appears on the integrator. A report will still be printed at the end of the run.

Selecting the Plot Quality

You may choose between two plot qualities—draft and presentation. The presentation plot is smoother than the draft plot and lags the real-time run. The HP 3396 uses the draft plot unless the presentation plot is specified.

1. **Press [OP()] [1] [ENTER].**
2. **Select the appropriate plot type or press [ENTER] to keep the current plot type.**

Example

```
INTEGRATION PLOT TYPE
(Source/Filtered/Unigram/No Plot)
ENTER PLOT TYPE [S/F*/U/N]: [ENTER]
Presentation plot [Y/N*]:
```

3. **Press [Y] [ENTER] for improved plot quality.**

   This example selects the presentation plot for the currently active method and will affect all plotting associated with this method. The presentation plot can also be selected in the [**PREP**] or [**EDIT**] [**METH**] dialog.

Setting the Plot Position

1. **Press [LIST] [ZERO] to list the current plot position and signal level.**

Example `* LIST: ZERO = 0, 0.0820`

The HP 3396 prints the *position*, *signal level*.

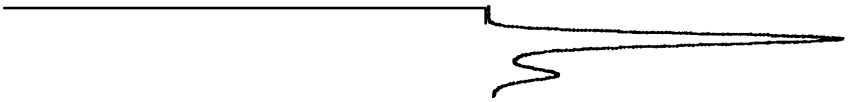
position is the location of the zero point on the plotter (left edge = −6; right edge = 100) or % of full scale deflection.

signal level is the value of the input signal (in millivolts) at the time the ZERO key is pressed. The integrator measures *signal level* when you press **[PLOT]** or **[START]**—then subtracts the measured value from all subsequent plotted points. This causes all plots to start at the same distance from the left side of the paper. This automatic zero-suppression affects only the plot, not the data being integrated.

2. **Press [ZERO] [−] [ENTER] to plot data without automatic zero-suppression.**
3. **Press [ZERO]** *position* **[ENTER] to set the baseline position on the plot**.

Example `* ZERO 50 @`

`START`



The integrator positions the plot about halfway across the page.

4. **Press [ZERO] [ENTER] to re-zero the plot during a run.**

Example

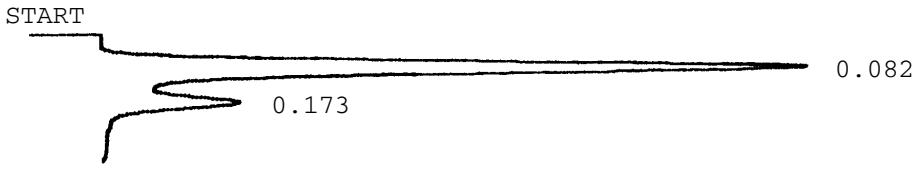
The HP 3396 takes a new reading of *signal level* and begins subtracting that value from the plotted points. The effect is to move the pen immediately to position and continue plotting. This does not affect data integration.

Setting the Chart Scale

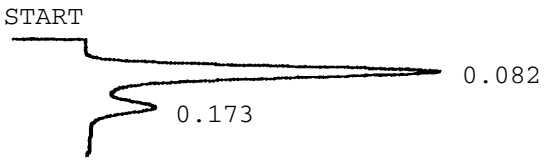
1. **Press [ATT 2↑]** *attenuation* **[ENTER] to set the chart scale.**

   attenuation is an integer from −8 to 36. Usually the attenuation is chosen to make the smallest peaks of interest readily visible in the chromatogram.

Example `* ATT 2^ 5 @`



Example `* ATT 2^ 6 @`



At attenuation = 0, the plotter sensitivity is approximately 1 mV full scale. Each step lower *doubles* the heights. Values may be changed while the plot is in progress. Each step higher *reduces* plotted peak heights by one half.

An entry of 36 turns the signal to the plotter off completely and is helpful when determining the zero point on the plot. This setting does not affect data integration.

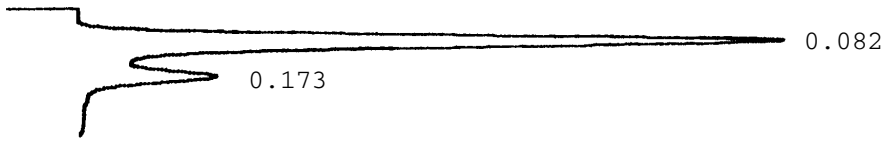
Setting the Chart Speed

1. **Press [CHT SP]** *chart speed* **[ENTER] to set the chart speed.**

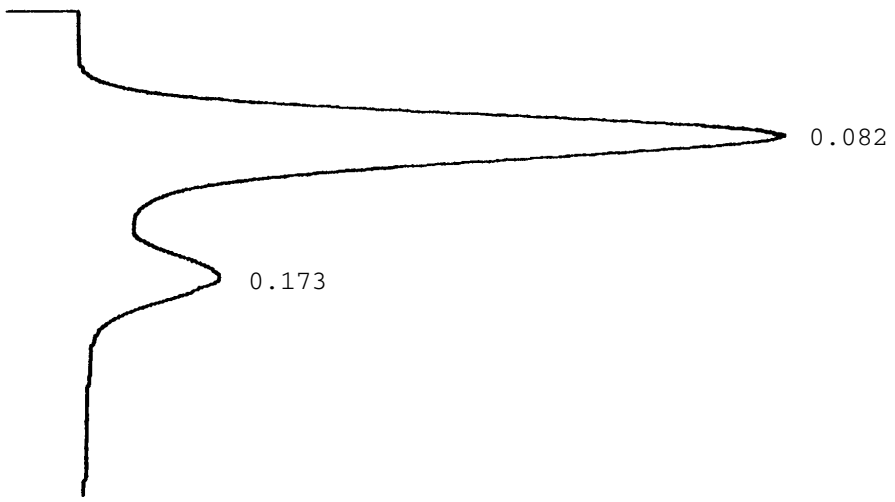
   c*hart speed* is a value between 0 and 30.0 cm/min, with a default value of 1 set when power is switched on. An entry less than 0.1 turns the chart drive motor off. The chart speed may be changed during a plot.

Example

```
*  CHT SP  5 @
```




```
*  CHT SP  20 @
```



Setting Form Size and Top of Form

The HP 3396 can be set for two sizes of paper.

1. **Position the paper with the printhead close to top of form.**

   See instructions in *Advancing the Paper* on the next page.

2. **Press [CTRL] and hold it while pressing [K] to set the form size at 66 lines (**USA letter size, 11 inches**)**.

   The top of form (TOF) is now set at the present position.

   or

3. **Press [CTRL] and hold it while pressing [V] to set form size at 72 lines (**ISO A4 size, 297 mm).

   The top of form (TOF) is now set at the present position.

Advancing the Paper

1. **Press [CTRL] and hold it while pressing [L] to advance paper to the next top of form.**

   The paper advances to the next top-of-form location, using the page length defined in *Setting Form Size* on the previous page.

2. **Press [CTRL] and hold it while pressing [A] to advance the paper 1/8 of a line.**

   This is useful when positioning the paper before setting the form size and top of form.

3. **Press [ENTER] to advance the paper one line.**

   Operates like a carriage return with an * printed out at the beginning of each line.

4. **Press [SHIFT] and hold it while pressing [ENTER] to advance the paper continuously.**

Setting Form Feed and Perforation Skipping Options

1. **Set the form size and top of form.**

   See instructions earlier in this chapter.

2. **Press [OP()] [5] [ENTER]**.

   Most of the items in this dialog concern the report and information to be included in it. These items are discussed in chapter 10. The last four items control paper feed during the plot and report and are discussed here.

Example

```
*  OP  # 5
PRINT & POST-RUN LIST OPTIONS

Large font [Y*/N]:                          See chapter 1
Store post-run report [Y/N*]:
External post-run report [Y/N*]:
List run parameters [Y/N*]:                 See
List timetable [Y/N*]:                      chapter 10
List calibration [Y/N*]:
List remote method [Y/N*]:

Form-feed before report [Y/N*]: Y [ENTER]
Form-feed after report [Y/N*]:  Y [ENTER]
Skip perforations in report [Y/N*]: Y [ENTER]
Skip perforations in plot [Y/N*]: Y [ENTER]
```

3. **Press Y for each form feed option to ensure that the report and plot are positioned at the beginning of a page**.

   The two form feed options cause an advance to the next top of page before and/or after printing a report.

4. **Press Y for both perforation skipping options to avoid printing on the perforated area between pages**.

   Skipping perforations in the plot may only be selected when perforation skipping in the report is also selected.

Understanding Time Programming

Time programming creates entries in the timetable. A printed code marks the execution time on the chromatogram.

	Key	Printed Code	See chapter
Chart Parameters	**[ZERO]** **[CTRL] [ZERO]** **[ATT 2↑]** **[CHT SP]**	ZE ^ZE AT CS	4 4 2 2
Integration Parameters	**[PK WD]** **[THRSH]** **[AR REJ]**	PW TH AR	3 3 3
Integration Functions	**[INTG( )]**	IF	3
External Events	**[EXT( )]**	EX	9
End of Run	**[STOP]**	ST	2

At the end of a run, parameters changed by the timetable revert to initial values, assuring that each run in a series begins with the same set of operating parameters. External events behave differently; see chapter 9 for details.

Making Timetable Entries

1. **Press [TIME]** *run time key* {*value*} **[ENTER]** to create a timetable entry.

   run time is the number of minutes after the run starts when the function is to occur; *key* is one of the time-programmable keys listed in the table; and *value* is the numeric entry associated with *key*, if there is one.

Example **[TIME]** [**.**] [**5**] [**CHT SP**] [**7**] [**ENTER**]

```
*  TIME  .5   CHT SP   7   @
```

This example sets the chart speed to 7 at thirty seconds into the run. Check the timetable entry by pressing
[**LIST**] [**TIME**] [**ENTER**]

```
*  LIST: TIME    @

0.500 CHT SP =  7.0
```

Timetable entries may also be made from the **[PREP]** or **[EDIT] [METH]** dialog.

Example **[EDIT] [METH] [ENTER]**

```
*  EDIT METH

1 = RUN PARAMETERS
2 = TIMETABLE EVENTS
3 = CALIBRATION FILE    etc

SECTION TO BE EDITED: [2] [ENTER]

TIMETABLE EVENTS
SELECT EVENTS FROM THE FOLLOWING MENU:

[IF/EX/ZE/^Z/AT/CS/AR/TH/PW/ST]
```

Codes for timed events; see previous page for corresponding keys

```
TIME:    [.] [5]  [ENTER]
EVENT:   [C] [S]  [ENTER]
VALUE:   [7 ]  [ENTER]
```

Deleting Timetable Entries

1. **Press [LIST] [TIME]** [**ENTER]** to list the current timetable.
2. **Press [DEL] [TIME] [ENTER]** to delete the entire timetable.

   or

3. **Press [DEL] [TIME]** *run time key* {*value*} **[ENTER]** to delete a specific timetable entry.

   or

4. **Press [DEL] [TIME]** *run time* **[ENTER]** to delete all entries at a particular *run time*.

   or

5. **Press [DEL] [TIME]** *key* **[ENTER]** to delete all entries of a specific *key*, regardless of *run time* or *value*, if any.

If *key* is **[INTG()]**, all entries for all function numbers will be deleted.

Example **[LIST] [TIME]** [**ENTER]**

```
*  LIST:  TIME   @

0.100 INTG # =  8
0.032 AR REJ =  66
0.500 CHT SP =  7.0
5.000 INTG # =  3
7.250 INTG # = -8

*  DELETE  TIME   INTG  #
*  LIST:  TIME   @

0.032 AR REJ =  66
0.500 CHT SP =  7.0
```

Listing and Editing the Timetable

1. **Press [LIST] [TIME]** {*run time*} [**ENTER]** to list the current timetable.

   If *runtime* is included, the listing starts at *runtime* and continues to the end of the table; otherwise, the entire table is listed. If there are no entries, the HP 3396 prints `EMPTY`.

EXT() entries are stored in a separate table; to list them, press

[LIST] [TIME] [EXT()] [ENTER] See chapter 9 for more information.

2. **Press [TIME]** *run time key* {*value*} [**ENTER]** to correct an entry in the current timetable.

   Assuming that the *run time* is correct, it will overwrite the previous entry for that function at that time.

Example **[LIST] [TIME]** [**ENTER]**

```
*  LIST:  TIME   @

0.032 AR REJ =  66

*  TIME  .032  AR REJ  100   @
*  LIST:  TIME   @

0.032 AR REJ =  100
```

Simultaneous Timed Events

Events scheduled at the same time occur in the order in which they were entered in the timetable. When two or more events are scheduled at the same time, only the code for the last event in the group is printed on the chromatogram.

See chapter 3 for related information about the priority of simultaneous integration events.

3

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Integrating and Reintegrating Data

Integrating and Reintegratng Data

Starting Integration

The HP 3396 integrates chromatographic information during a sample run in real-time and during reintegration.

To start real-time integration

1. **Be sure the appropriate signal or data source is connected to the integrator.**
   See the *Reference* manual for installation instructions.
2. **Set [ZERO], [ATT2 ^], and [CHT SP] or use default values.**
   See chapter 2 for more information.
3. **Set [AR REJ], [THRSH], and [PK WD] or use default values.**
   Instructions for choosing these values are discussed later in this chapter.
4. **Select a plot type for the chromatogram or use the default filtered plot.**
   See chapter 2 for more information.
5. **Press [OP()] [2] to store data for later use.**
   See chapter 7 for more information.
6. **Start the run. [START]**
   Real-time integration produces processed peak data for a report. The data may be stored for later use. Signal data may also be stored for later reintegration.
7. **Stop the run. [STOP]**
8. **Inspect the chromatogram and report.**
   Make appropriate adjustments in run parameters if necessary. See chapter 10 for more information about report reading.

Checking Parameter Values

1. **Press [LIST] [LIST] to list the currently selected run parameters.**

Example

```
*  LIST: LIST
PEAK CAPACITY:  1244      number of peaks integrator can currently store

ZERO     =  0,  -0.828
ATT  2^  =  0
CHT SP   =  1.0
AR REJ   =  0
THRSH    =  0
PK WD    =  0.04
```

Use the default values listed above if you are unsure of the best values to use for your run. All run parameters may be adjusted during real-time integration or reintegration to optimize presentation, peak detection, or quantitation.

Individual parameters may also be listed separately.

Example [**LIST**] [**PK WD**] returns only the current peak width value.

```
*  LIST: PK WD  =  0.01
```

See chapters 2 and 4 for related information.

The presentation of the chromatogram is controlled by

- [**ZERO**] the baseline zero position
- [**ATT 2^**] attenuation
- [**CHT SP**] chart speed.

These parameters are discussed in Chapter 2.

The quantitation of the chromatogram is controlled by

- [**AR REJ**] area reject
- [**THRSH**] threshold
- [**PK WD**] peak width.

These parameters are discussed in this chapter.

Selecting Peak Width Values

The peak width run parameter specifies the expected widths (in minutes) of peaks at approximately half-height. If no peak width is selected, the integrator uses a default value of 0.04 minutes, suitable for many analyses.

1. **Press [PK WD]** *value* **[ENTER] to set peak width.**

Example `* PK WD .01 @`

Use the table below to choose an appropriate peak width *value* for your application.

Peak Width Range	Application
0.01 to 0.05 minute	High-resolution capillary or packed GC columns
0.04 to 0.2	High-performance LC, moderate length packed GC columns
0.15 to 0.6	High-performance LC, long packed GC columns
0.5 to 2.5	High-performance LC, low efficiency GC
2.5	Low-pressure (column) LC, some types of amino acid analysis, nonchromatographic peak integration

2. **Press [LIST] [PK WD] to list the current value for peak width.**

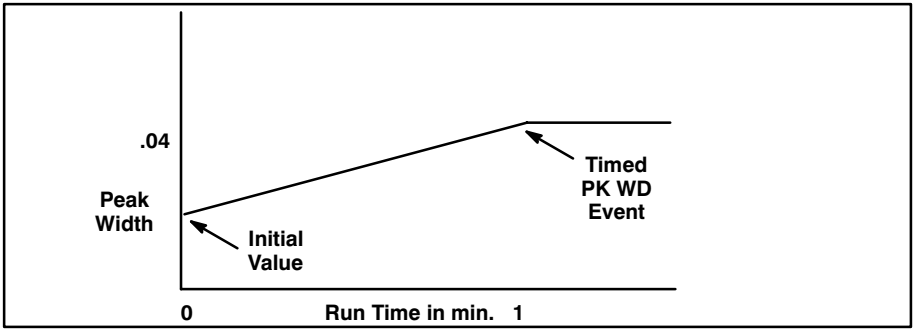
Changing Peak Width Settings During a Run

If peak widths are almost constant through a run, as they usually are with temperature programming or gradient elution, a single PK WD value will suffice for the entire run. If the widths change significantly, as in isothermal or isocratic runs, a peak width profile can be generated by time-programmed changes.

1. **Press [TIME]** *run time* **[PK WD]** *new value* **[ENTER] to time-program a peak width change.**

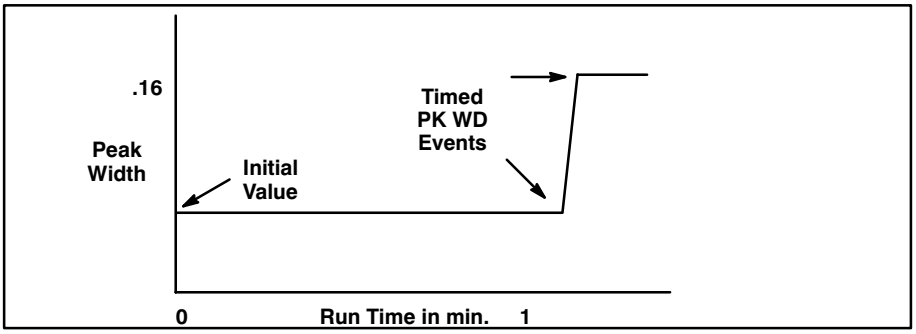
The peak width parameter behaves differently from all other time-programmed values. The change to *new value* is not immediate. Instead, the value changes in a smooth ramp from the time of the previous value.

Example `* TIME 1.00 PK WD .04 @`



To make a step change, use two timed events close together.

```
*  TIME 1.00  PK WD  .04  @
*  TIME 1.01  PK WD  .16  @
```



See chapter 4 for related information.

Selecting Threshold Values

The peak detection threshold [THRSH] is a value representing the signal level below which the HP 3396 considers all baseline deviations as noise. A peak with height less than the Threshold value is ignored.

There are two ways to select threshold values.

1. **Press [THRSH]** *value* **[ENTER] to set threshold.**

value is an integer from −6 to 28. The values are a binary series; an increase of 1 unit doubles the minimum height that will be accepted. The minimum value (−6) is equivalent to two height counts. One height count represents 1/8 microvolt.

Example `* THRSH 5 @`

2. **Press [THRSH] [ENTER] to set auto-threshold.**

The HP 3396 then measures the signal noise and assigns an appropriate value to Threshold. Auto-THRSH determinations must be performed when the chromatographic signal is stable and peak-free, as during a blank (no sample) run or while using PLOT, using the Peak Width value (or profile) selected for the analysis. The process takes 5 x Peak Width minutes and the Ready LED blinks during the determination. Auto-threshold can be automatically built into a run by programming INTG() 6. See instructions for the integration functions later in this chapter.

Threshold has two side effects. High values delay the decision whether to accept or reject a given peak. This delays the printing of the start tick mark on the chart, so that it may only approximate the position of the real start of peak. Increasing Threshold also causes solvent peaks to terminate earlier.

3. **Press [THRSH] [−] [ENTER] to abort auto-threshold.**

4. **Press [LIST] [THRSH] to list the current value for threshold.**

Selecting Area Rejection Values

Each peak must have an area count above the area rejection value to be reported or stored in the processed peak file.

1. **Press [AR REJ]** *value* **[ENTER] to set the area reject limit.**

value is an integer representing area counts in 1/8 μV-seconds.

Example `* AR REJ 800 @`

As a convenience, area reject may also be entered in scientific notation using “E-format”.

[AR REJ] [**1**] [**E**] [**6**] [**ENTER**]

is the same as

[AR REJ] [**1**] [**0**] [**0**] [**0**] [**0**] [**0**] [**0**] [**ENTER**]

2. **Press [LIST] [AR REJ] to list the current area rejection value.**

Using the Integration Functions

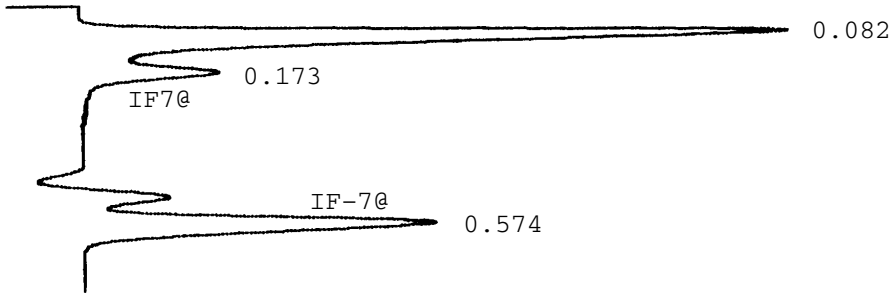
The integration functions customize baseline construction when the standard construction is not appropriate. Some functions have both active and inactive (canceled) states; others are single events.

Integration Number	What it does
INTG( ) **0**	Set baseline now
INTG( ) **1**	Set baseline at next valley
INTG( ) **2**	Set baseline at all valleys
INTG( ) **3**	Process next peak as a solvent peak
INTG( ) **4**	Turn off automatic solvent detection
INTG( ) **5**	Draw horizontal baseline
INTG( ) **6**	Measure and update Threshold
INTG( ) **7**	Turn off retention time labeling
INTG( ) **8**	Turn on Start/Stop marks
INTG( ) **9**	Turn off integration
INTG( ) **10**	Increment Threshold
INTG( ) **11**	Invert negative peaks
INTG( ) **12**	Clamp negative peaks
INTG( ) **13**	Show functions 11 and 12
INTG( ) **14**	Start peak sum window

Integration functions may be entered while the run is in progress or time-programmed to occur at a specified run time.

1. **Press [INTG()]** *function number* **[ENTER] to activate an integration function during a run.**

Example



Integration function 7 is turned on after the second peak so that retention times for subsequent peaks are not printed until the function is turned off again.

2. **Press [INTG()] [−]** *function number* **[ENTER] to cancel an integration function during a run.**

Time-Programming Integration Functions

When the **[START]** key is pressed to begin a run, all integration functions are inactive.

1. **Press [TIME]** *run time* **[INTG()]** *function number* **[ENTER] to activate an integration function.**

To have a function active at the start of a run, enter the activating command in the Timetable at time 0.

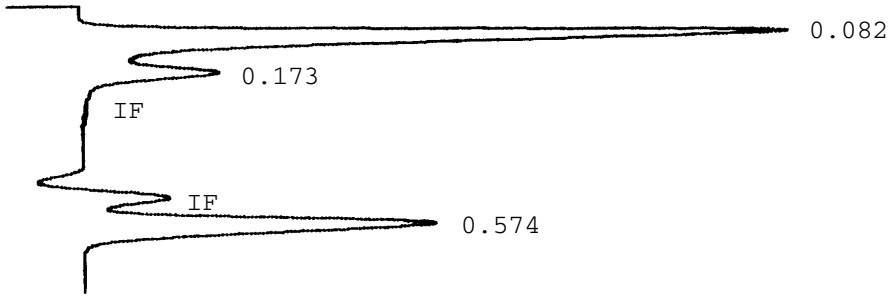
When a timed event is to apply to a particular peak, the execution time must be after the retention time (peak apex) of the preceding peak but before the retention time of the target peak.

2. **Press [TIME]** *run time* **[INTG()] [−]** *function number* **[ENTER] to cancel an integration function.**

The notation `IF` is printed on the chart when any integration function is executed.

Example

```
*  TIME  .3   INTG #   7   @
*  TIME  .55  INTG #  -7   @
```



Priority of Baseline Functions

When more than one baseline function is in effect at the same time, a priority order is applied.

Priority of Baseline Functions

Highest priority ↓	INTG() 0	Set Baseline Now
	INTG() 5	Extend Baseline Horizontally
	INTG() 1	Set Baseline at Next Valley Point
	INTG() 2	Set Baseline at All Valley Points
Lowest priority	INTG() 3	Skim from Next Peak

Example

```
*  LIST:  TIME   @
0.100 INTG # =  0
0.100 INTG # =  1
0.100 INTG # =  5
```

Thus INTG() 0 will *always* set the baseline to the current signal value, but the entry to set baseline at the next valley point (INTG() 1) will be ignored if the baseline is being constructed horizontally (INTG() 5).

Reintegration

Reintegration is the process of reanalyzing signal data from storage. The data used in reintegration can be either from a real-time run or a previous reintegration.

1. **Press [OP()] [2] [ENTER] to store raw or bunched run data from a real-time run or a reintegration.**

Caution

Storing bunched signal data, processed peak data, or report data from the reintegration of a signal data file always overwrites the previous set of reintegration result files from that same signal data file, if there were any. Change the input signal data filename before the reintegration to save the previous copies.

If you specify storage of signal data for a reintegration and then attempt to reintegrate a .BNA file from the same device as that specified for storage, the signal data will *not* be stored, because that would overwrite the input file. The reintegration will proceed, but the message

```
Error storing signal; filespec
FILE ALREADY OPEN
```

will be printed afterward. Either change the name of the input signal file to something else (**COPY** or **RENAME** commands; see *Using Application Programs* to automate this process) or use option 2 to disable signal data storage or change its destination. A similar conflict arises with local run time storage when signal data files are being sent to a host computer. See chapter 7 for more information.

2. **Make a real-time run or reintegration.**

3. **Change the method, if desired.**

The HP 3396 uses the integration parameters present in internal memory at the time the **ANALYZE** command is typed, except for sample information which is stored with the signal data.

To select the integration parameters

- Do nothing if the ones in memory are correct.
- Use key commands to make any desired change.
- Load a new method with a new set of parameters (see chapter 8).

4. **Type AN**{**ALYZE** *filespec* **, I** } **to begin reintegration.**

Only the first 2 letters are required; everything else is optional.

filespec names the storage device, file name, and file name extension where the raw or bunched data to be reintegrated is stored. See chapter 7 for more about *filespec*. If *filespec* is omitted, the HP 3396 selects a default file:

- If this is the first reintegration after a real-time run, the default is the signal data file stored during that run. If no data was stored, the default is undefined.
- For the second and later reintegrations, the default is the signal file analyzed in the previous reintegration.

Use the **I** parameter if you change some of the integration parameters but want to ensure that the original PK WD profile (which is stored with the data) is used.

If the device specified, either in *filespec* or as a default, is H, and the host computer link is operating in MUTE Mode, the **ANALYZE** command will fail. The message

```
INVALID MUTE HOST CONVERSATION
```

will appear. MUTE Mode is the default computer link mode at power-on. The host computer must be programmed to change the HP 3396 to VOCAL Mode, and to supply the called-for signal data file, for reintegration to succeed.

During reintegration the HP 3396 reanalyzes (reintegrates) run data produced by a prior real-time run or reintegration. As in real-time integration, the integrator plots a chromatogram, quantitates the data, and reports the results. Reintegration stops when the HP 3396 reaches the end of the signal in the file, when [**STOP**] is pressed, when a TIMETABLE STOP occurs, when a remote control STOP occurs, etc.

Example To produce a report with sample information (ISTD AMT, SAMP AMT, MUL FACTOR) different from that in the signal data file:

1. **Press OP()] [4] [ENTER] to suppress printing of the post-reintegration report.**

```
*  OP # 4

REPORT OPTIONS
Suppress local report [Y/N*]: Y [ENTER]
```

2. **Press OP()] [7] [ENTER] to enter the new sample information.**

```
*  OP # 7

DEFAULT SAMPLE INFORMATION
USE SAMPLE TABLE IN MANUAL RUN [Y/N*]: Y [ENTER]

ISTD AMT  [0.0000E+00  ]:          Enter new information here
SAMPLE AMT  [0.0000E+00  ]:
MUL FACTOR  [1.0000E+00  ]:
                          the dialog continues...
```

3. **Type AN{ALYZE, I } to begin reintegration.**
4. **Press the [REPORT] key to generate a report.**

Example To produce a report with a calculation type *different* from that in the signal data file:

1. **Press [OP()] [4] [ENTER] to suppress printing of the post-reintegration report.**

```
*  OP # 4
REPORT OPTIONS
Suppress local report [Y/N*]: Y [ENTER]
```

2. **Press [PREP] or [EDIT] [CALIB] [ENTER] to change the calculation type and create the calibration table.**

```
*  PREP CALIB  @

E = EXTERNAL STANDARD
I = INTERNAL STANDARD
N = NORMALIZATION

CALIB PROCEDURE  [E*/I/N]:        Enter new calculation type; see Chapter 5 for details
REF % RTW  [   5.000]:
NON-REF % RTW  [   5.000]:

RF BASED ON AREA OR HEIGHT [A*/H]:
CAL#     RT           AMT             NAME
   1  :                              Enter calibration table; see Chapter 5
```

3. **Type AN{ALYZE** *filespec*, **I } to begin reintegration.**

4. **Press [REPORT] to generate a report with the new calculation.**

Reintegration Tips

Reintegration can be many times as fast as the real-time run. To achieve the highest possible speed

- Store and reintegrate bunched data.
- Use the largest PK WD value which still gives accurate results.
- Either suppress the plot (fastest) or select a Unigram plot.
- If you need a conventional plot, keep CHT SP less than 0.3/PK WD to keep the plot from slowing down the process.

4

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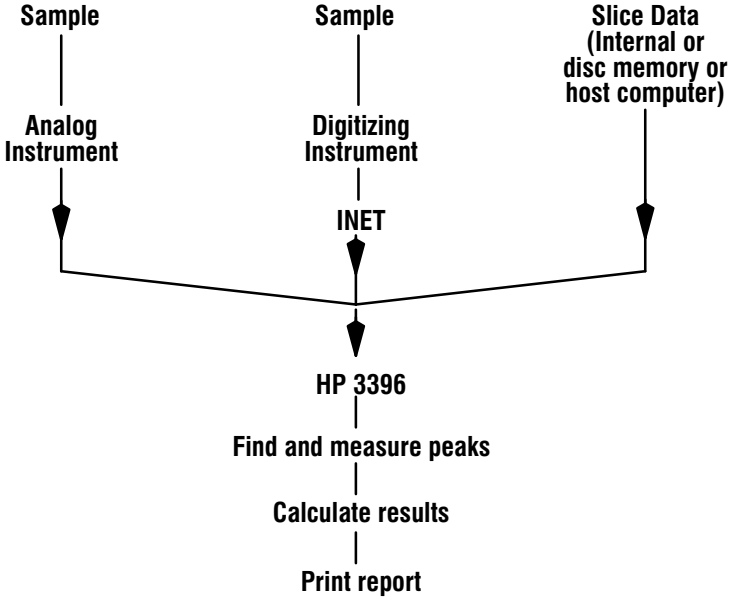
Understanding Integration

Understanding Integration

Understanding How the Integrator Integrates

This chapter describes the internal HP 3396 peak detection and measurement operations.

The HP 3396 processes an analog (voltage) signal from a gas or liquid chromatograph or other analytical instrument. It can also process digitized signals from instruments on the Instrument Network (INET). Finally, it can process data from its own internal memory, from disk storage and from a host computer.



The integrator takes the following steps to find and measure peaks

1. Accepts data
2. Prepares the data for integration
3. Searches the prepared data for peaks
4. Measures the peaks

5. Constructs the chromatographic baseline
6. Corrects the peak measurements for baseline
7. Saves data
8. Calculates amounts for the report (see chapters 5 and 6)

The rest of this chapter discusses these topics in this order. Each discussion is divided into

- The simplest case
- One or more complexities
- Integration Functions, which are deliberate interferences with normal signal processing, are noted if any apply. The details of the functions are deferred to the end of the chapter.

Data Acceptance

From an Analog Instrument

The instrument produces a voltage that varies with time. The HP 3396 converts this to a series of digital numbers, each representing one “area slice”. An area slice is bounded by the start time of the slice, the start time plus the slice width, the analog (voltage) signal, and a reference voltage.

The HP 3396 uses a slice width of 0.05 seconds (20 slices per second) and an electrical reference of approximately 0 volts. The reference is not related in any way to the “correct” baseline; it is simply a voltage lower than any we expect to see from the instrument.

Data collection begins when the **[START]** key is pressed and ends when the **[STOP]** key is pressed or a time-programmed STOP event occurs.

From an INET Instrument

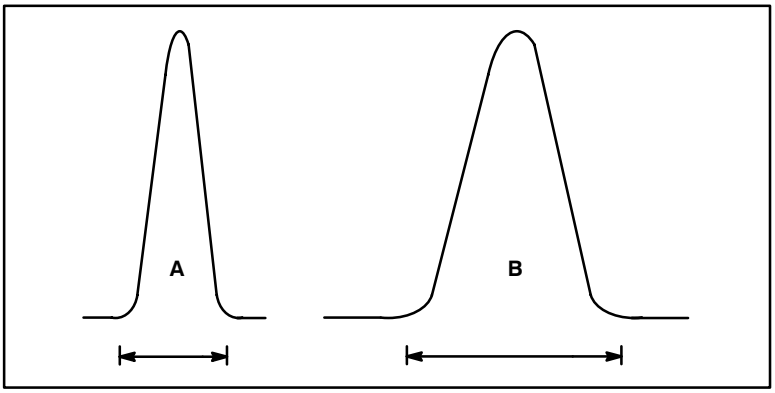
Some instruments, such as the HP 5890 Gas Chromatograph, can produce an area slice signal and transmit it to the HP 3396 via the INET network. Since the signal is already digital, the conversion process just described is not needed. The HP 3396 processes the full range of signal data from the HP 5890 GC.

From a Memory File

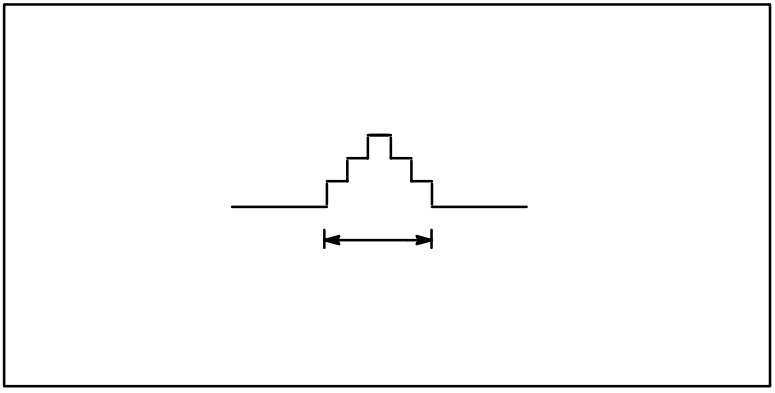
The HP 3396 can accept and process data from files in its own memory, or data stored on a disc memory associated with the HP 3396 or a host computer. This is usually done to reintegrate, using different parameters, data that originally came from the HP 3396.

Data Preparation

Peaks are detected by passing the signal, which is now a stream of area slices, over an internal pattern or template. When the match is good, we have a peak; when it isn't, we don't. There is, however, a complication. Consider this pair of peaks

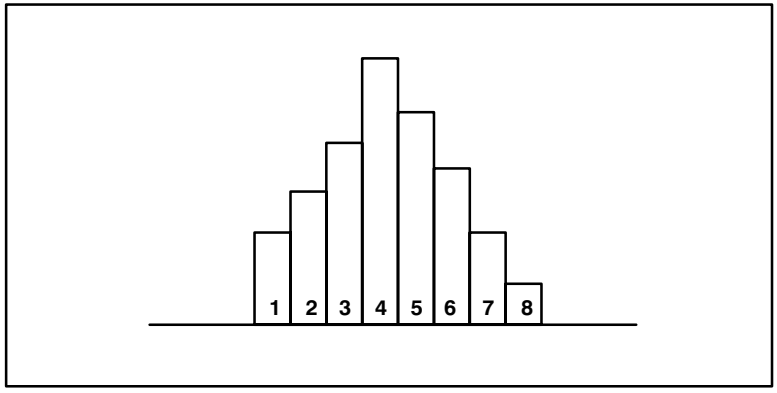


and this simple peak template

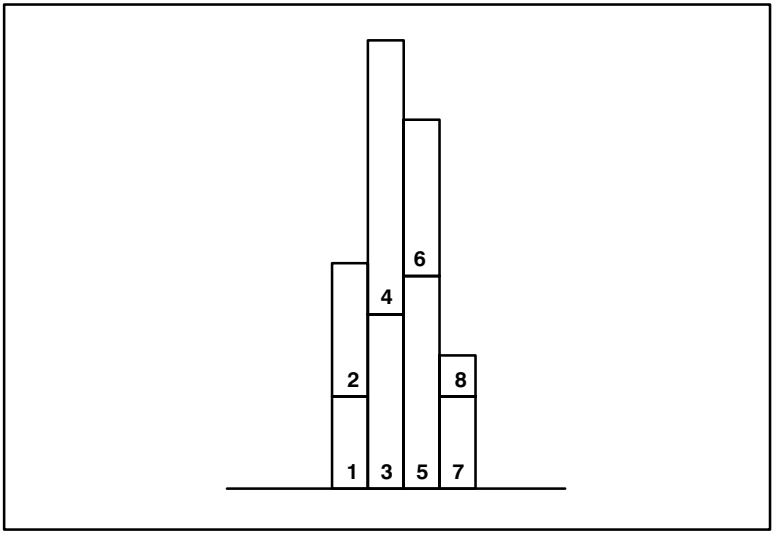


The template is a good width match for peak A, but not for peak B. Even when it is perfectly centered on peak B, there is a great deal of "peak character" which is outside the template. This reduces the sensitivity at the center of peak B and broadens the response as the signal passes the template. The result is to make peak B much harder to detect than peak A.

It's clear that the problem is caused by a width mismatch between Peak B and the template. There are two ways to correct this—widen the template or narrow the peak. It turns out to be much easier to narrow the peak. Here's a broad one, represented as a numbered series of area slices:



If this peak is too wide for a good match with the filters, we can easily narrow it by adding pairs of slices together. The result is:



This “bunched” peak has the same area as the original but is narrower and higher and easier to detect. Both the retention time and the height have changed, but since we know what we did, it’s easy to correct them.

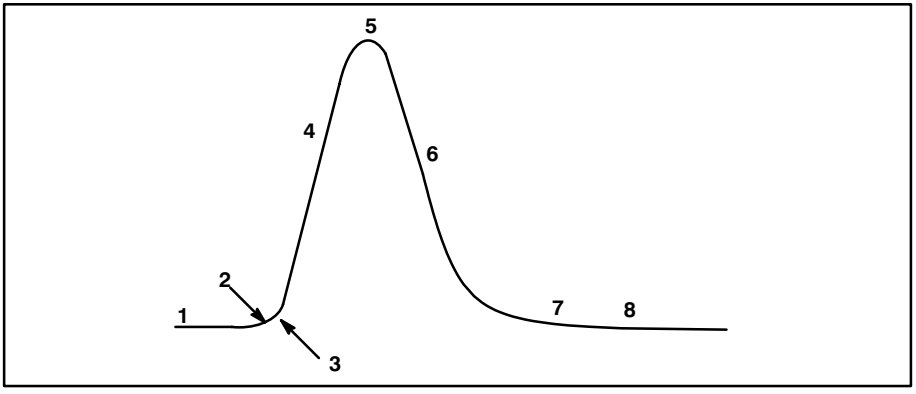
Peak Recognition

There are two parts to the search for peaks. Part 1 rejects random noise in the signal based on a rough measure of height. Part 2 compares the signal exceeding the threshold height with a set of internal templates to find regions that have peak-like characteristics.

A peak with height less than Threshold is ignored.

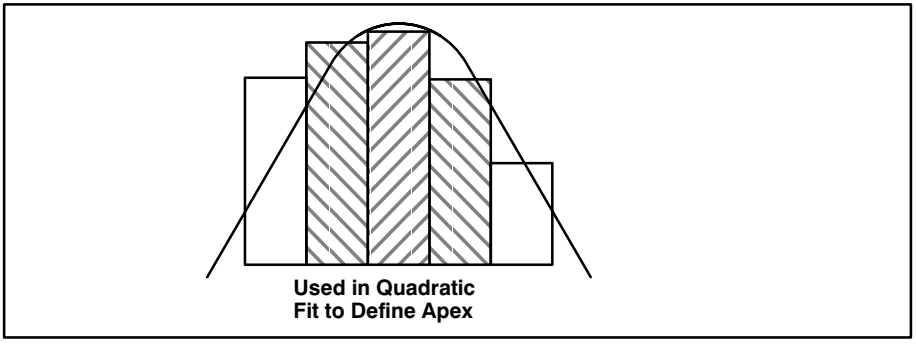
The Simple Case—Isolated Peaks

As the integrator scans the data, it examines the slope (difference between successive slices) and curvature (positive or negative). So long as these remain within preset bounds, this is baseline. If the bounds are exceeded, a peak may be starting. If the condition persists, the integrator decides that it is on the upslope of a peak. A complete, isolated peak looks like:



1. Slope and curvature within limits – track baseline
2. Slope and curvature above limits – perhaps a peak?
3. Slope remains above limit – we've got a peak!
4. Curvature becomes negative – front inflection point
5. Slope becomes negative – top of the peak
6. Curvature becomes positive – rear inflection point
7. Slope and curvature within limits – approaching end of peak
8. Slope and curvature remain within limits – end peak, track baseline

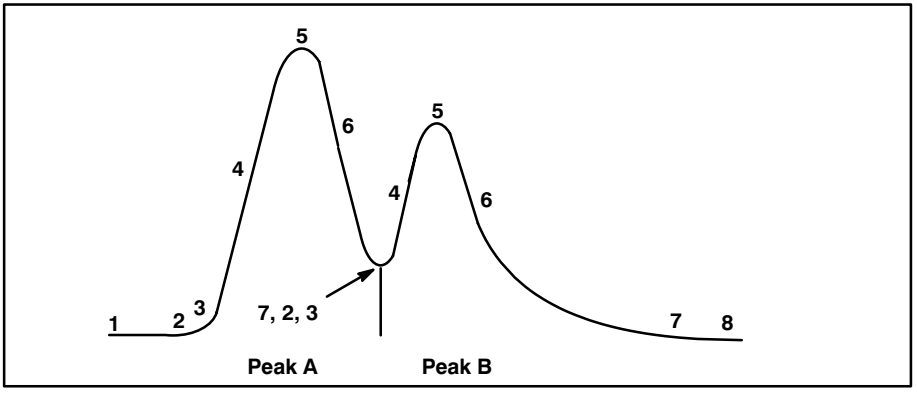
Step 5 identifies the approximate top of the peak. The HP 3396 uses the tallest slice and one slice on either side, fits them to a quadratic equation, and solves the equation to find the retention time and peak height.



Peak end is found using a formula based on the retention time of the peak, its measured width, the rate at which it approaches baseline, and for solvent peaks, the present Threshold value. This has been tested against many peaks and provides a reasonable end-of-peak decision.

Complexity 1—Merged Peaks

A peak may follow all the steps to 7 but then begin to rise again. It is merged with the following peak so that there is no baseline between them. The HP 3396 responds by forcing the first peak to end at the lowest point and then integrating the second one. This repeats as many times as necessary when we have a cluster of merged peaks.



The lowest (valley) point is located in much the same way that the peak top is found. The HP 3396 uses the smallest slice and its two neighbors, fits the data to a quadratic, and solves for the minimum.

Related Integration Functions

1. Set baseline at next valley.
2. Set baseline at all valleys.

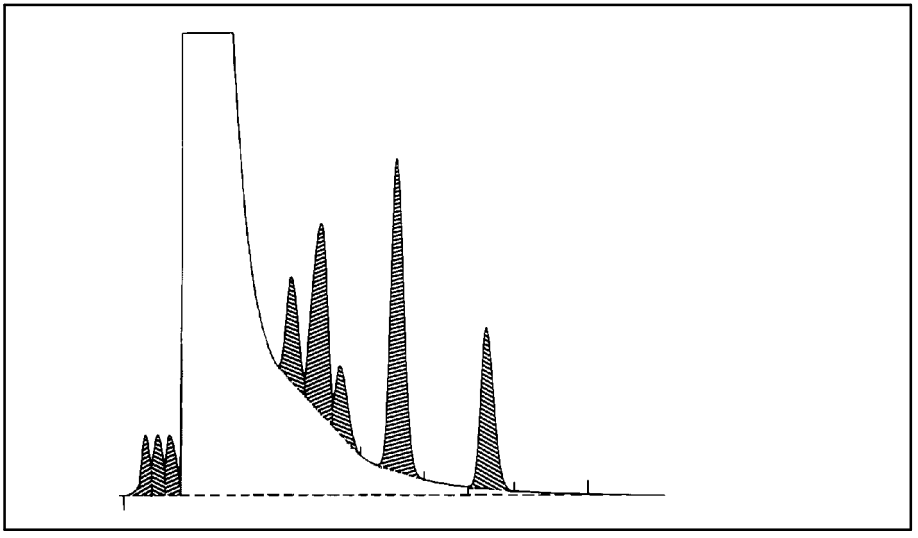
See *The Integration Function Description*s later in the chapter for related information.

Complexity 2—Solvent Peak Detection

The HP 3396 measures the difference in height of successive slices on the front side of a peak. If this difference exceeds 16 millivolts for analog input or 800 millivolts for INET, the peak is treated as a solvent peak.

If this results in nonsolvent peaks being treated as solvents, integration function 4 may be used to prevent testing. Function 3 marks a peak which does not meet the slope criterion as a solvent.

Peaks on the downslope of a solvent peak are called riders and will be integrated relative to a tangent baseline which is drawn from the start of the peak and is tangent to the solvent downslope. The point of tangency is called a Tangent Point.



Related Integration Functions

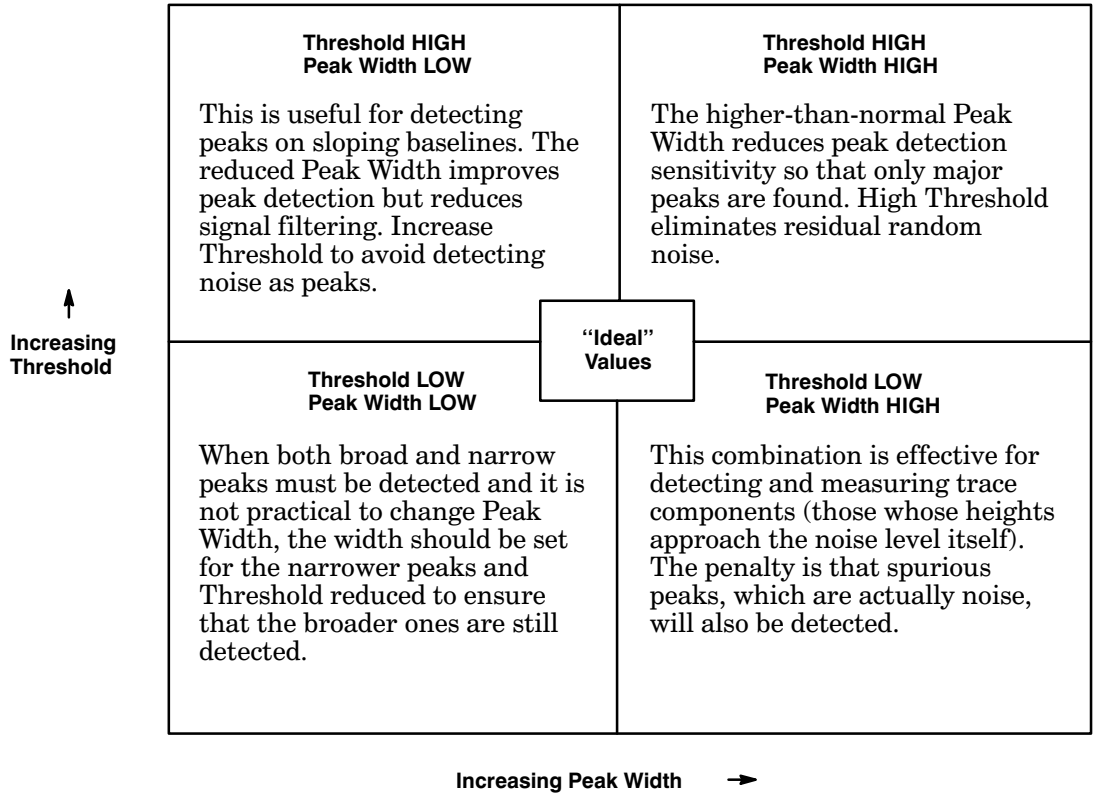
3. Skim from next peak
4. Disable autotangent skimming

See *The Integration Function Descriptions* later in the chapter for related information.

Optimizing Peak Recognition

The best conditions for recognizing isolated, symmetric peaks on a flat, quiet baseline is to match the Peak Width parameter to the measured width of the peaks at half-height. The auto-Threshold value is appropriate for eliminating noise.

When peaks cluster together or the baseline slopes or is noisy, these ideal values must be modified. The figure shows how to modify these values appropriately.


Threshold HIGH
Peak Width LOW
This is useful for detecting peaks on sloping baselines. The reduced Peak Width improves peak detection but reduces signal filtering. Increase Threshold to avoid detecting noise as peaks.
Threshold HIGH
Peak Width HIGH
The higher-than-normal Peak Width reduces peak detection sensitivity so that only major peaks are found. High Threshold eliminates residual random noise.
"Ideal" Values
Threshold LOW
Peak Width LOW
When both broad and narrow peaks must be detected and it is not practical to change Peak Width, the width should be set for the narrower peaks and Threshold reduced to ensure that the broader ones are still detected.
Threshold LOW
Peak Width HIGH
This combination is effective for detecting and measuring trace components (those whose heights approach the noise level itself). The penalty is that spurious peaks, which are actually noise, will also be detected.
Increasing Threshold
Increasing Peak Width

Tips For Selecting Peak Width Values

1. When peaks are large compared to the noise in the signal, a useful rule of thumb is

   PK WD must be
   MORE THAN 1/4 OF
   BUT LESS THAN 2 TIMES
   the actual width.

2. When peaks are very small or when noise is high, and particularly when both conditions occur simultaneously, it may be necessary to "overfilter" the signal to detect the peak. This is done by using a larger-than-normal PK WD value; however, if PK WD is too much greater than the actual width of the peak, the peak itself may be filtered out. This situation requires some experimentation to find the most appropriate value.

3. The report of analysis contains a column headed WIDTH. These numbers are good approximations of the widths of peaks at half the peak height. If the HP 3396 fails to detect peaks that are clearly present, examine the WIDTH values for the peaks that are found. Use this information and inspection of the chromatogram to select a more appropriate value for PK WD.

4. The Unigram plot can be used to select an appropriate value or profile (discussed next) for the PK WD parameter.

If the PK WD value (or profile) is a good match to the actual peak widths, all of the peaks in the Unigram will have the same width. The heights are then proportional to the peak areas.

The Unigram transformation is

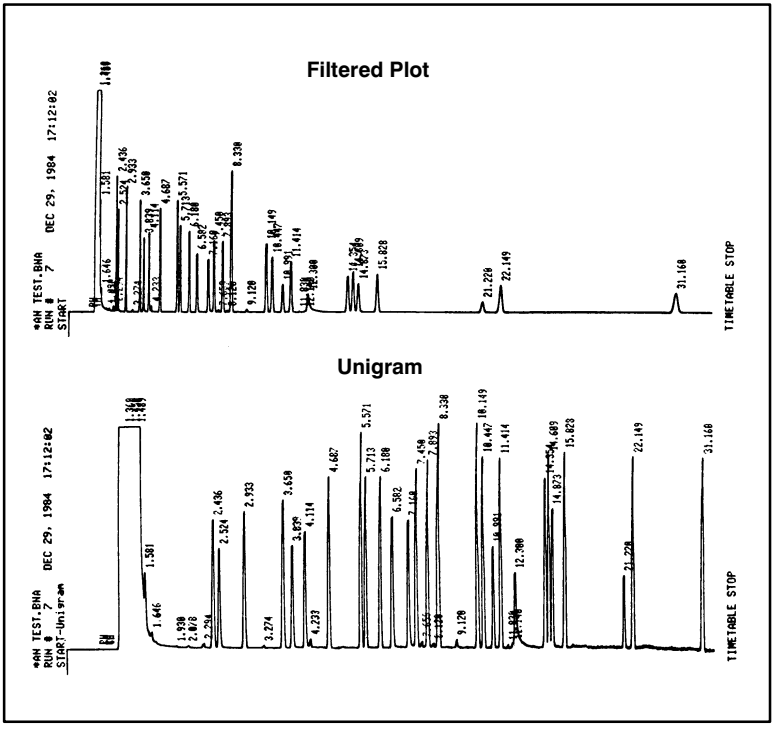
Time axis: Replace linear time with time divided by the peak width.

$$\frac{0.1 \times \text{CHT SP}}{\text{PK WD}}$$

Height axis: Replace linear height with height times peak width.

$$100 \times \text{PK WD} \times (\text{filtered peak height})$$

These two figures show the same run data as a filtered plot and as a Unigram.



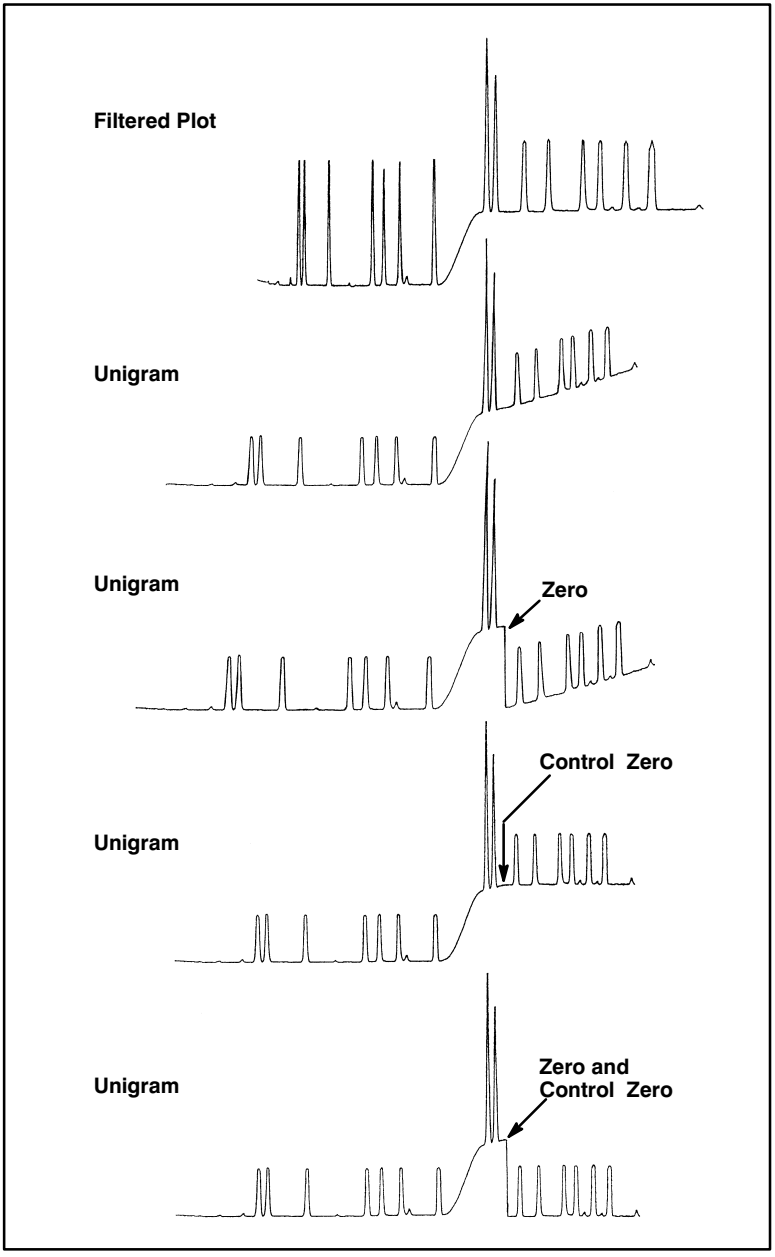
A displacement of the signal may appear in a Unigram as a shift followed by severe baseline drift. These two effects have different causes, and separate commands are provided to deal with them.

1. **Press [ZERO] [ENTER] to reset the baseline to the present value of the signal.**

The effect is to move the baseline to the left of the chart.

2. **Press and hold [CTRL] while pressing [ZERO] then release both and press [ENTER] to remove the drift caused by the signal displacement.**

These commands can be used separately or together, and both may be time-programmed.

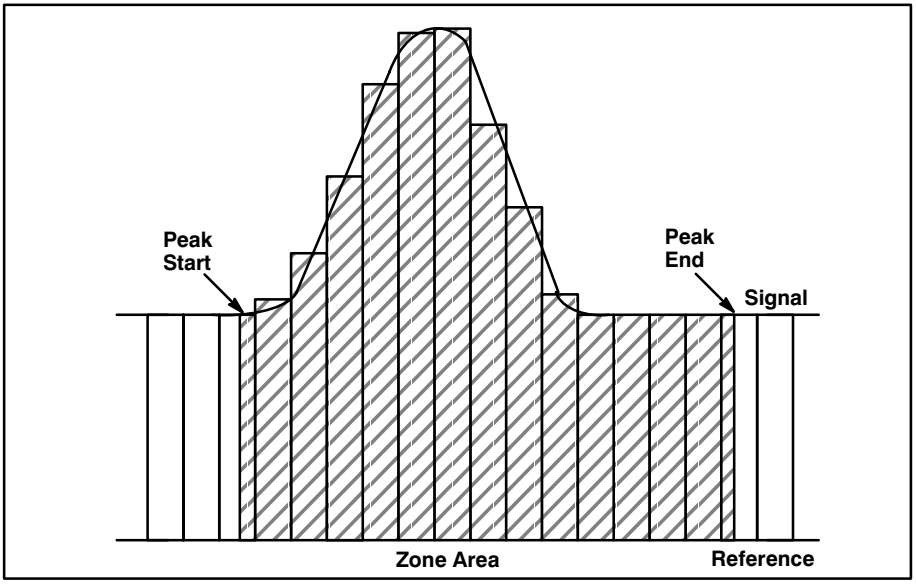


See Chapter 3 for related information.

Peak Measurement

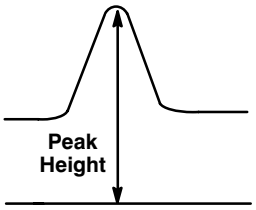
Area

Measuring peak areas is trivial once the Start of Peak, End of Peak, Valley Point, and any Tangent Points have been located. Vertical lines from each of these Cardinal Points are dropped to an electrical reference level to create a series of zones. For each zone, all the area slices are added within it. If a Cardinal Point falls inside a slice rather than on a slice boundary, the slice area is divided between the two adjacent zones according to where the point is within the slice. The result is shown below.



Height

The vertical distance is measured from each peak apex to the reference level.

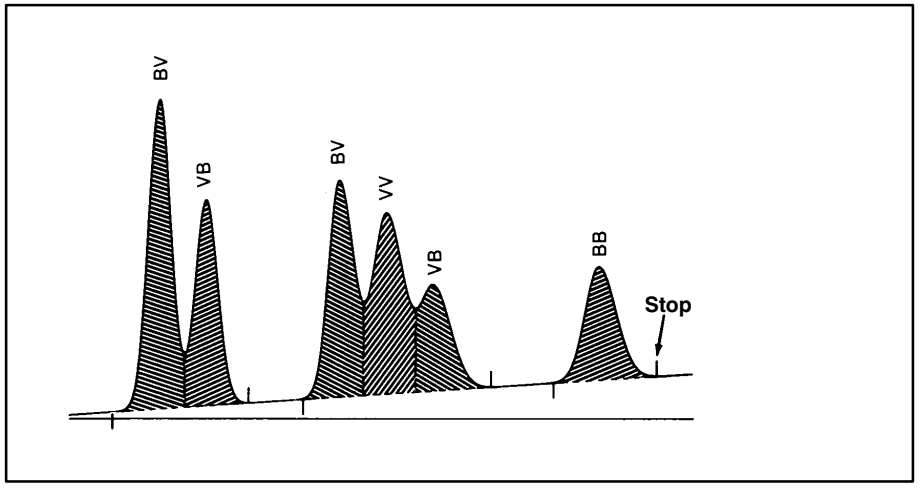


Chromatographic Baseline Construction

The Simple Case—No Solvents, No Timed Events

The baseline is a continuous series of straight line segments that connect these points:

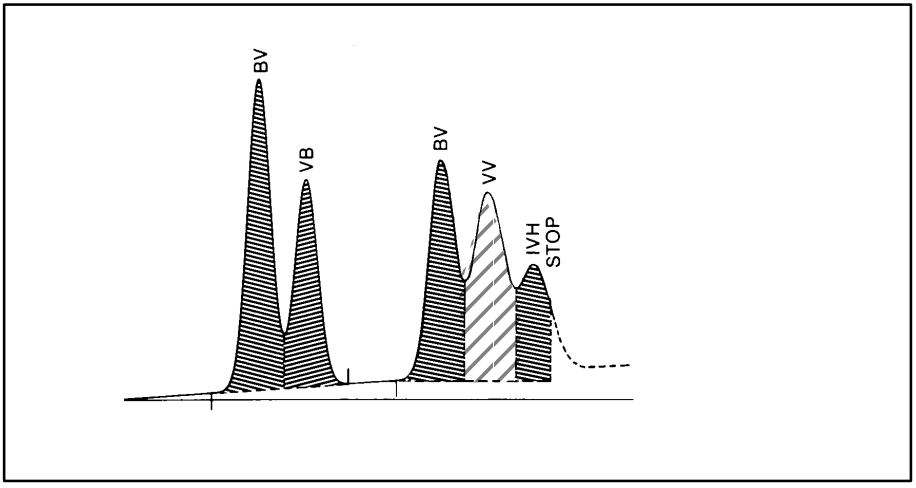
1. The signal level at the START of the run.
2. The start and end of peaks or merged groups. These points are marked by large tick marks (downscale for start, upscale for end) on the chart and by the letter B in the start or end position in the TYPE column of the report.
3. The signal level when STOP occurs, if it happens when no peak is in progress.



Complexity 1—STOP During a Peak

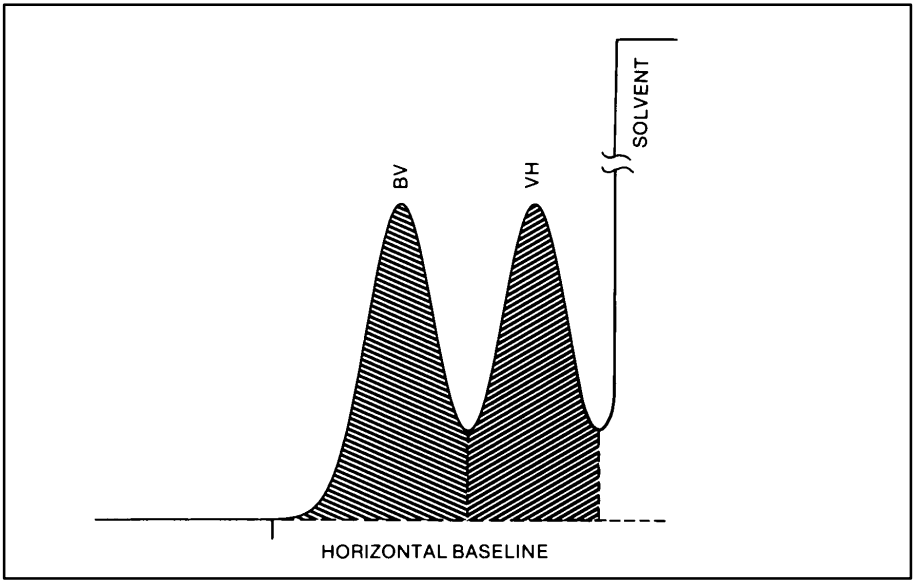
If the STOP occurs before the apex of the peak, the peak is not reported.

If it occurs after the apex, the last segment of the baseline is a horizontal line drawn from the last declared baseline point to the STOP time, with a dropline from the signal at this point. The peak type in the report has I (for incomplete) in the warning column and H (for horizontal extension) in the end column.

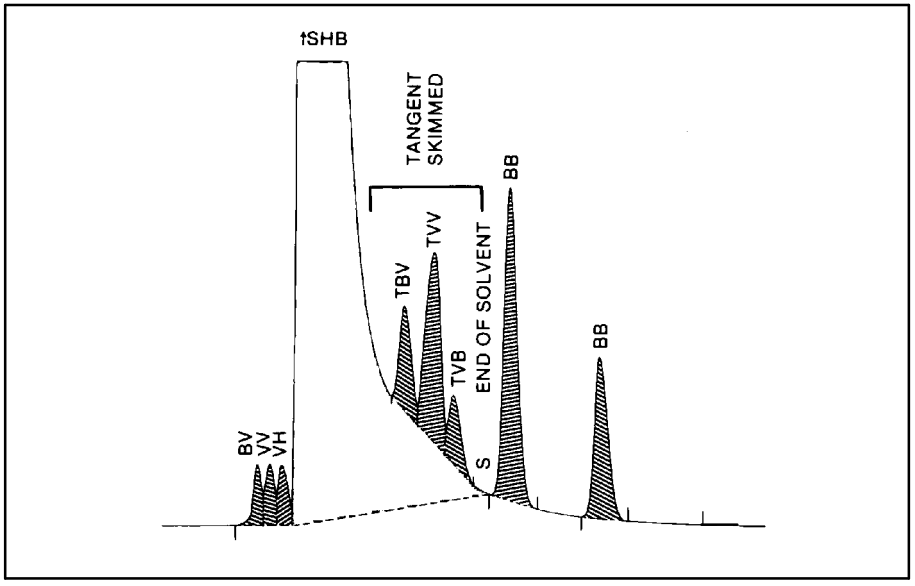


Complexity 2—Solvent Peaks

If a solvent peak starts above baseline, the segment before the solvent is a horizontal extension from the last declared baseline point to the start of the solvent peak. The peak TYPE in the report has S in the solvent column and H in the start column.



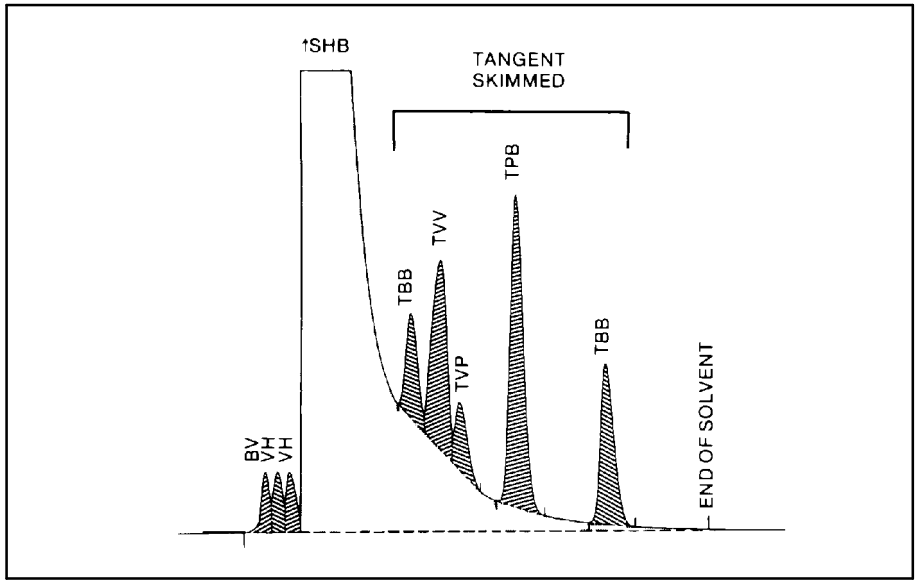
Solvent peaks normally end using the same test as for ordinary peaks. They will end sooner if the start, end, or valley point of a rider peak is below the Threshold level in effect. Baseline is set to the signal level at the point, the solvent ends, and tangent skimming ceases. The peak is marked with an S on the chart to indicate that it ended due to THRSH level.



See *Understanding Reports* in Chapter 10 for related information.

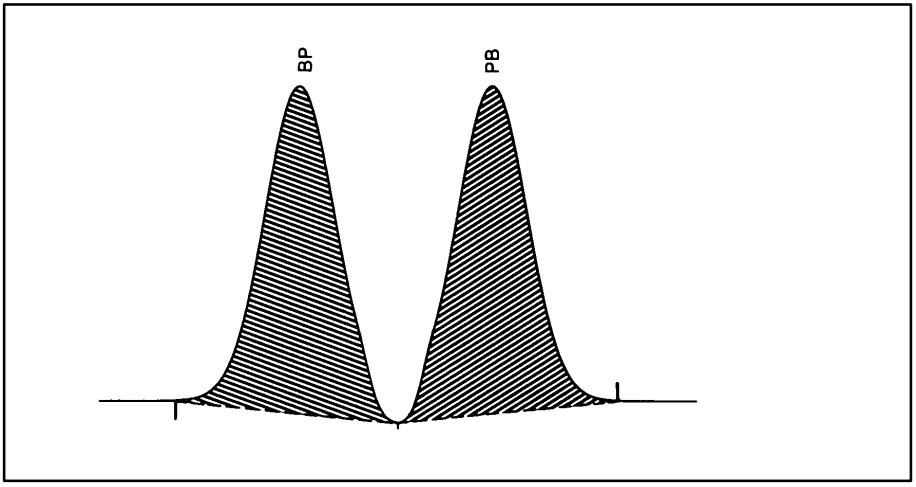
Complexity 3—Rider Peaks

A local baseline tangent to the tail of the solvent is created for rider peaks. All such peaks are marked by small tick marks. The TYPE column must be consulted to determine which points are local baseline points and which are local valley points. All rider peaks are TYPE T in the solvent column. The local baseline follows the same rules as the overall baseline.



Complexity 4—Baseline Penetration

If the signal drops below the baseline that has been constructed using these rules, this is a baseline penetration. The HP 3396 redraws the baseline to eliminate the penetration.



Detection of penetration is quite sensitive, often producing P codes for peaks that appear to be on perfectly normal, flat baselines. If a penetration is invisible on the chromatogram relative to the least significant information being measured, the effect of redrawing the baseline can be ignored.

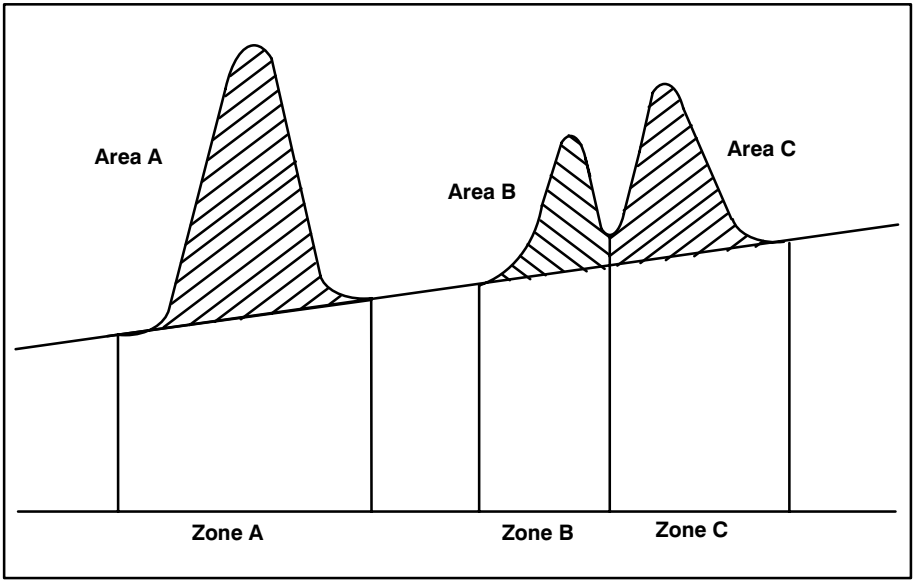
Integration Functions Related to the 4 Complexities

1. Set baseline now
2. Set baseline at next valley
3. Set baseline at all valleys
4. Skim from next peak
5. Extend baseline horizontally

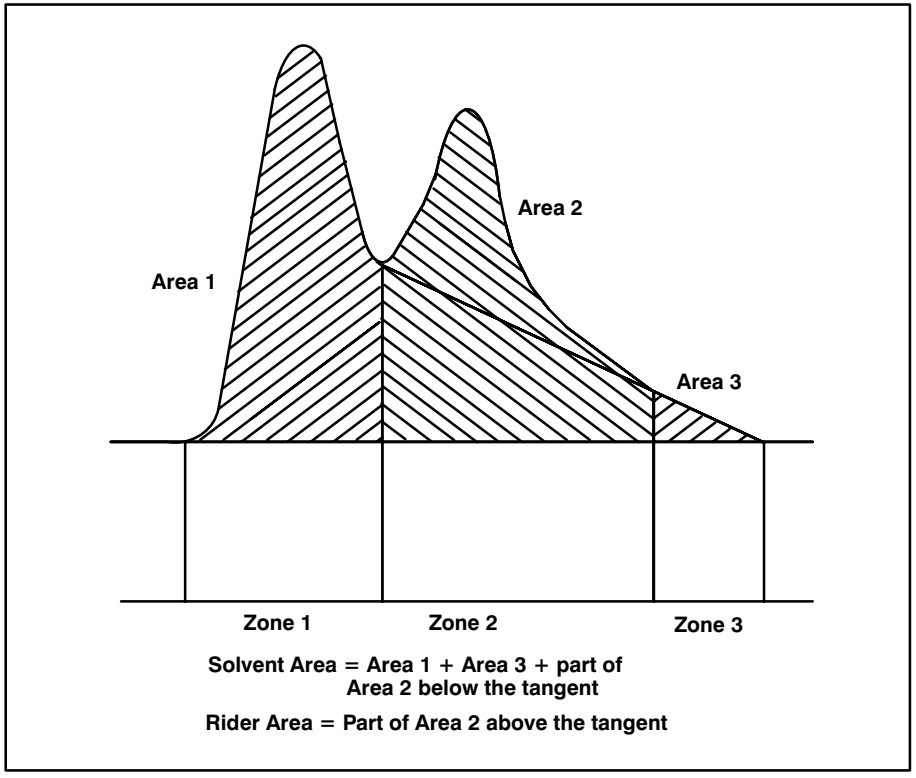
See *The Integration Function Description*s later in the chapter for related information.

Baseline Corrections

Zone areas and peak heights have been measured relative to an electrical reference level that is not related to the chromatographic baseline. We now know where the real baseline is for every zone. Compute the area (a trapezoid) between the baseline and the reference level, subtract, and have the baseline-corrected areas. A similar calculation yields the baseline-corrected peak height.



Rider peaks on a solvent tail require a more elaborate calculation. Zones 1 and 3 are corrected in the way just described. Zone 2 requires some extra calculation to divide the area above baseline. The part above the tangent line is reported as the rider peak; the rest is summed with the corrected areas from Zones 1 and 2 to give the solvent area.


Area 1
Area 2
Area 3
Zone 1
Zone 2
Zone 3
Solvent Area = Area 1 + Area 3 + part of Area 2 below the tangent
Rider Area = Part of Area 2 above the tangent

Peak Data Storage

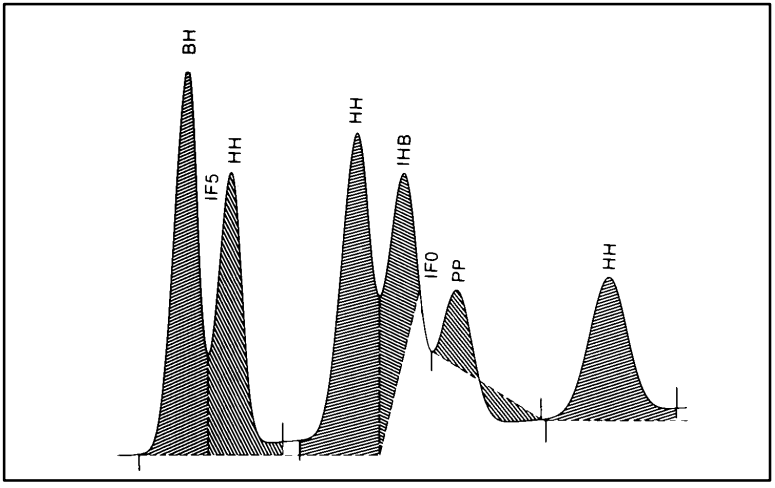
Data is stored for calculations for each peak with area greater than the specified Area Reject amount. This peak data includes the retention time in minutes, the area in 1/8 microvolt-seconds, and the height in 1/8 microvolts.

The Integration Function Descriptions

INTG() 0 Set Baseline Now

INTG() 0 causes an immediate reset of the integrator baseline to the current value of the signal. It is useful where a baseline shift occurs during a run, such as after a valve switch.

Example In the figure, INTG() 0 terminates the fourth peak.

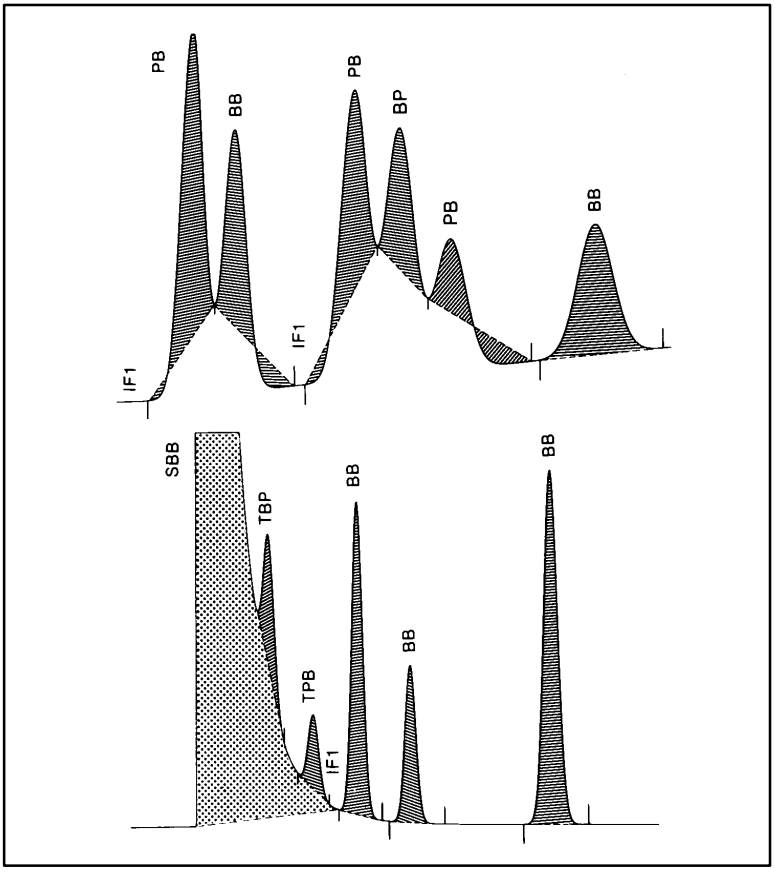


If INTG() 0 is activated while tangent skimming peaks from a solvent tail, both tangent skimming and the solvent peak are terminated.

INTG() 1 Set Baseline at Next Valley Point

INTG() 1 causes a baseline reset at the next valley point encountered then cancels itself automatically. If the valley point is on a solvent downslope, both tangent skimming and the solvent peak are terminated.

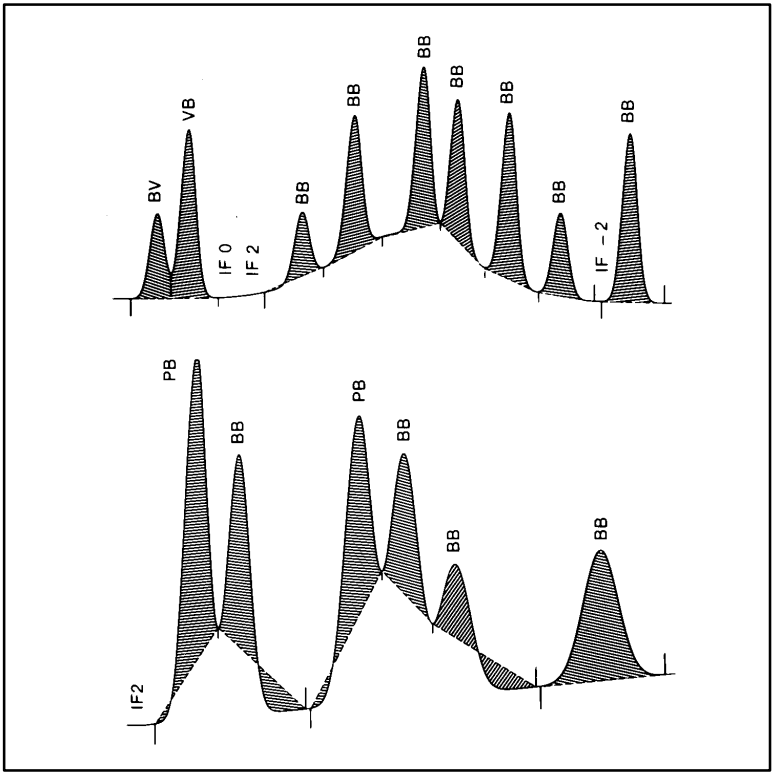
The **[INTG()] [−] [1]** command is provided to cancel the reset command if the expected valley should not occur, which would cause the reset to occur at a later valley. Thus the two commands should be used as a pair bracketing the expected valley time.



INTG() 2 Set Baseline at All Valley Points

The INTG() 2 function forces the baseline to pass through all valley points until it is canceled. It may be used to lift a series of peaks off a broad swell in the baseline.

This repeated resetting of the baseline can cut off corners of peaks. Such corners become “negative area”; they reduce the total measured area of the peaks.

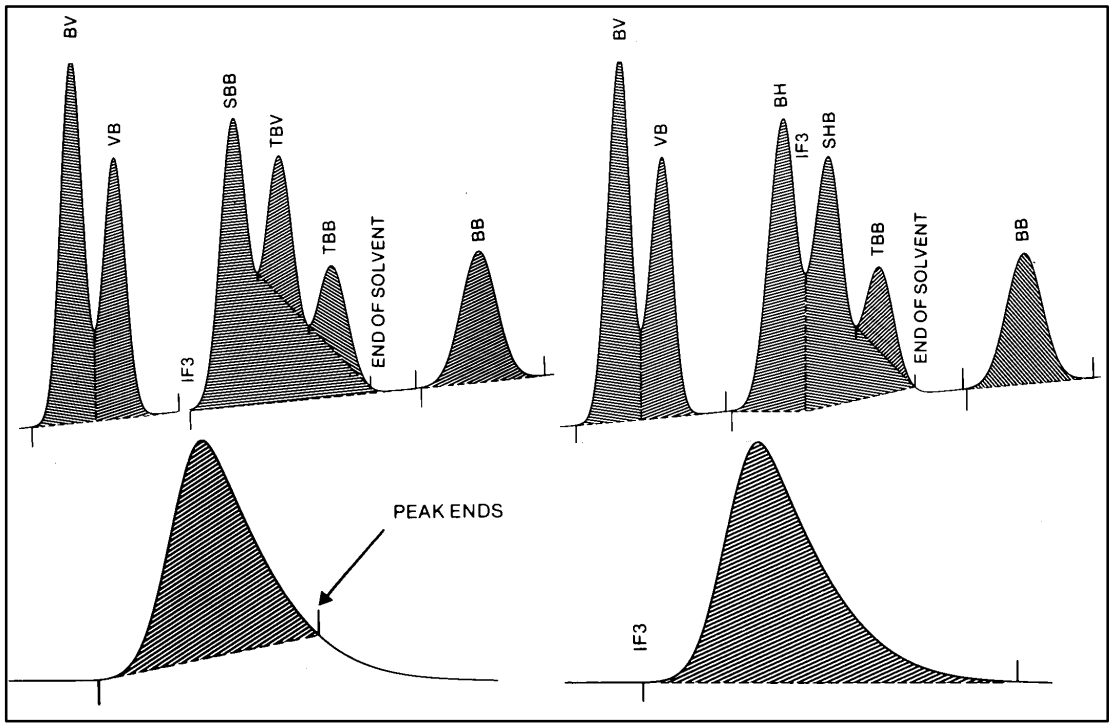


INTG() 3 Skim from Next Peak

A solvent peak may rise too slowly to be recognized automatically, or there may be a group of peaks well into the run that you feel should be treated as a solvent with a set of riders. This usually involves a merged group of peaks where the first one is far larger than the rest. The simple dropline treatment would exaggerate the later peaks because they are actually sitting on the tail of the first one. By forcing the first peak to be recognized as a solvent, the rest of the group is skimmed off the tail.

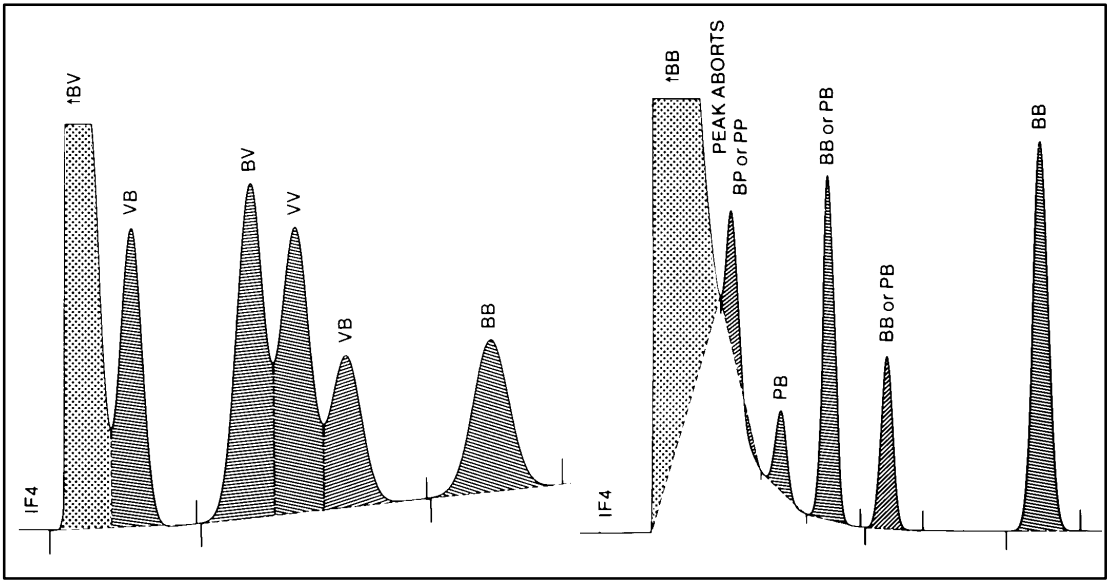
INTG() 3, when executed before the apex of a chosen peak, designates the peak to be a solvent. Tangent skimming is performed on peaks on the downslope of this peak. Skimming continues until the solvent peak terminates or another INTG() baseline function is executed.

INTG() 3 is useful for isolated peaks that tail badly. The peak may be terminated and baseline reset before all area under the tail is measured. Declaring a peak to be a solvent turns off normal termination tests, allowing area to be accumulated for a longer time.


BV
VB
IF3
SBB
TBV
TBB
END OF SOLVENT
BB
PEAK ENDS
BV
VB
BH
IF3
SHB
TBB
END OF SOLVENT
BB
IF3

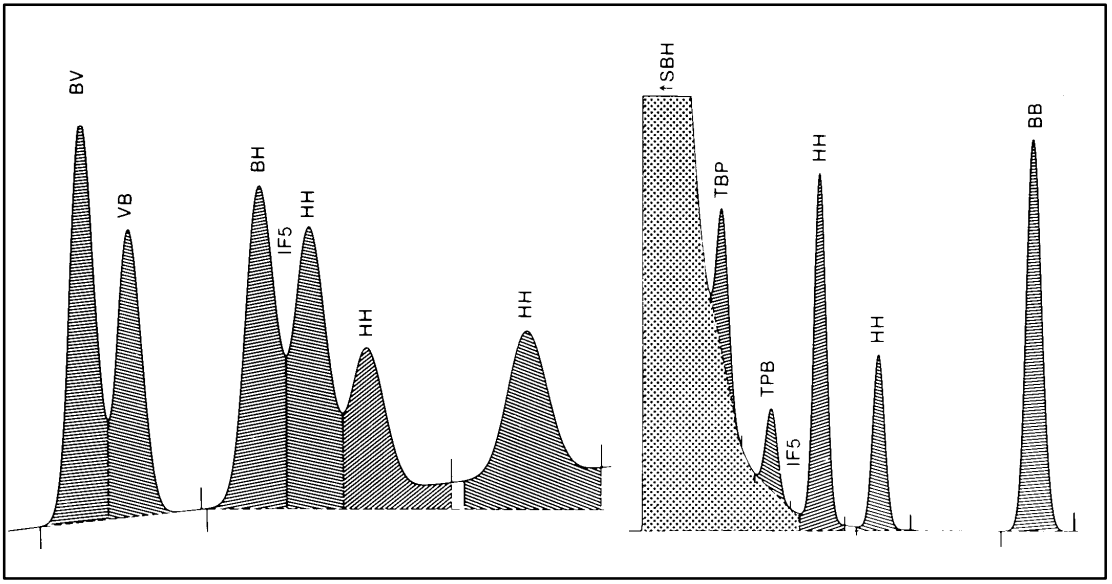
INTG() 4 Disable Auto-Tangent Skimming

INTG() 4, executed before the start of a peak, disables solvent testing. The function can be used if component peaks of interest have upslopes steep enough to be detected incorrectly as solvents. INTG() 4 restores normal operation.



INTG() 5 Extend Baseline Horizontally

INTG() 5 extends baseline horizontally from the last declared baseline point. If the signal penetrates the horizontal baseline, the baseline is reset to the lowest point, then continued horizontally. The construction continues until canceled by INTG() 5.



INTG() 6 Measure and Update Threshold

INTG() 6 performs the same function during a run as the **[THRSH] [ENTER]** command does in Plot mode or the Ready state. **[INTG()] [−] [6] [ENTER]** stops the measurement with no change in Threshold if it is entered before the measurement is completed.

The auto-THRSH process may be repeated during a run by time programming Integration Function 6. If a step change is wanted, Integration Function 10 may be used.

INTG() 7 Turn Off Retention Time Labeling

INTG() 7 , when activated, suppresses printing of retention times on the chromatogram. INTG() 7 resumes printing.

INTG() 8 Turn On Start and Stop Tick Marks

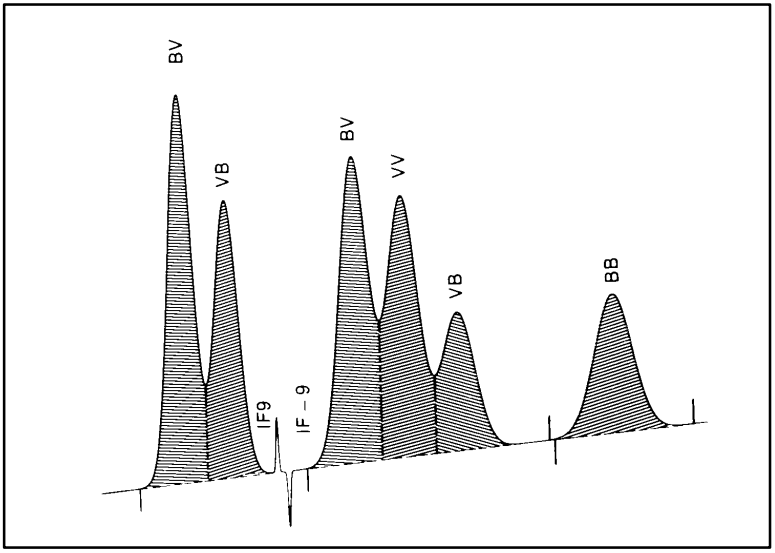
INTG() 8 , when activated, causes printing of peak start and stop tick marks on the chromatogram. INTG() 8 cancels printing. The integration plot type (option 1) must be filtered for tick marks to be activated.

INTG() 9 Turn Off Integration

INTG() 9 is useful for skipping parts of the chromatogram where the signal goes negative, or eliminating baseline disturbances caused by changes made to the chromatographic system, such as valve switching.

INTG() 9 turns integration OFF. Baseline is determined as if a STOP had occurred. The chromatogram plot continues and the Timetable remains active.

INTG() 9 resumes integration. The signal value at this time is used as a baseline point.



INTG() 10 Increment Threshold

INTG() 10 increases the Threshold value by 1; INTG() 10 decreases it by 1.

INTG() 11 Invert Negative Peaks

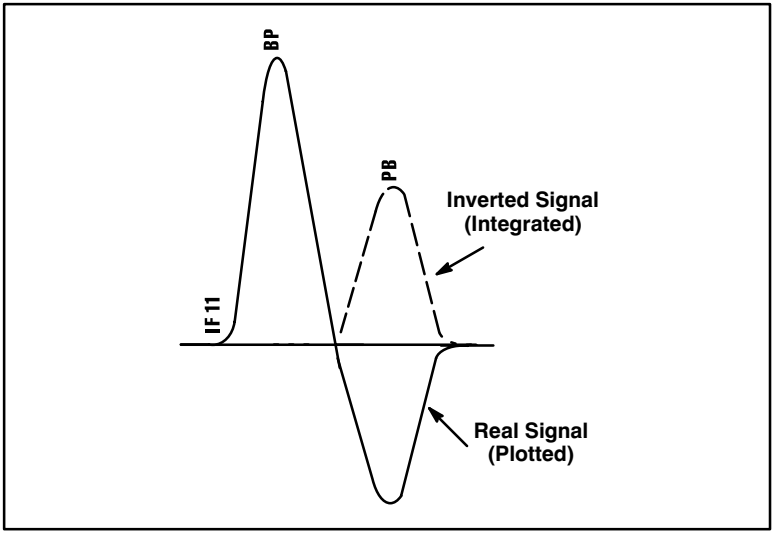
INTG() 11 inverts negative peaks so that they can be processed by the peak recognition and measurement software. Peaks that have been inverted are marked with an N in the TYPE column of the report. Negative peak inversion continues until canceled by INTG() 11.

When INTG() 11 is activated, and if neither INTG() 11 nor INTG() 12 is active before the event, an Inversion Level is set at the last baseline point declared. If either INTG() 11 or INTG() 12 is active at the time of the event, the existing Inversion Level is used.

All signal values below the Inversion Level are inverted over that level. The inversion equation is

Signal Out = Inversion Level + ABS [Signal In—Inversion Level]

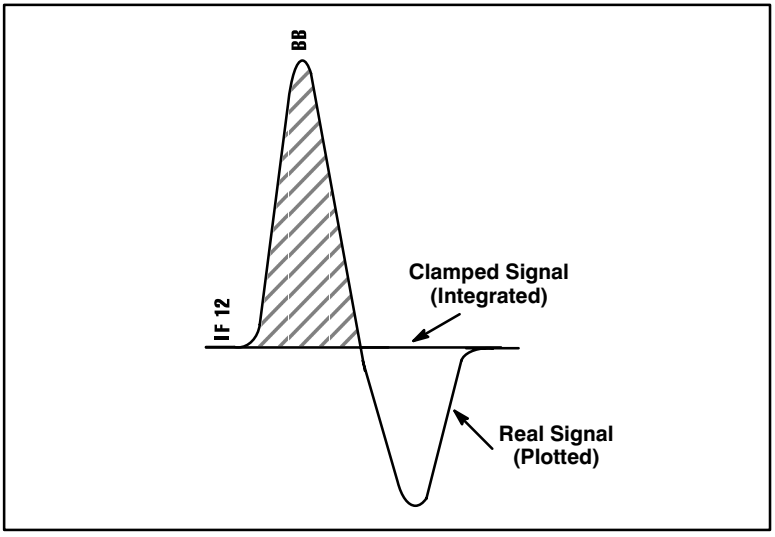
where ABS means the absolute (unsigned) value.



Negative peaks that extend beyond the −10 mV limit of the integrator will *not* be integrated.

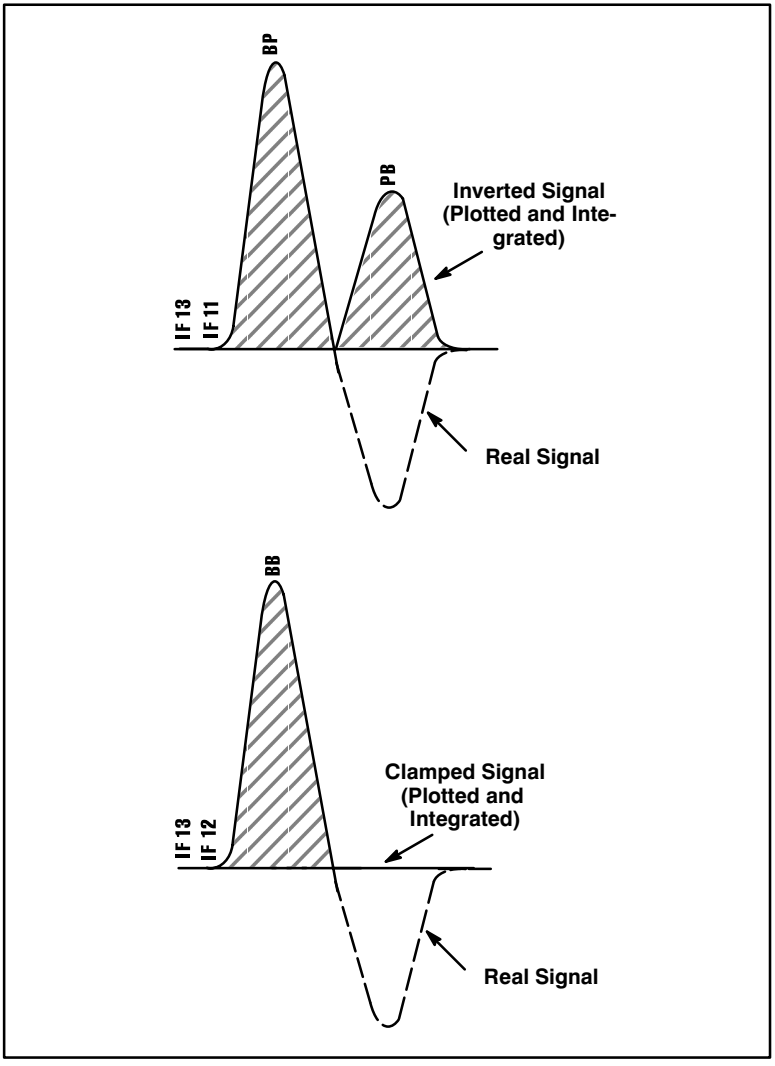
INTG() 12 Clamp Negative Peaks

INTG() 12 is similar to INTG() 11, except that signal values below the Inversion Level are raised to that Level. The effect is to ignore signal excursions below that level. Clamping continues until cancelled by INTG() 12.



INTG() 13 Show Functions 11 and 12

When INTG() 13 is inactive, the chart shows the signal without the effects of INTG() 11 or 12. The inversion or clamping is not visible. With INTG() 13 active, the chart shows the signal as it appears to the integration software.



INTG() 14 Start Peak Sum Window

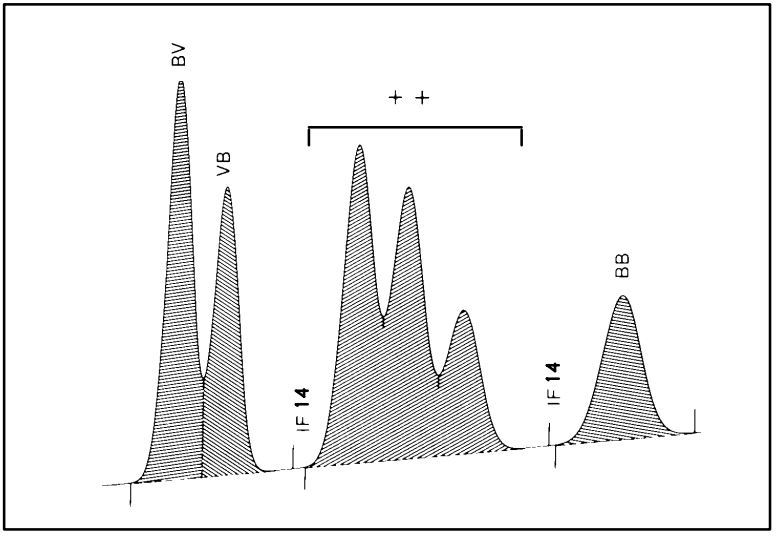
INTG() 14, when activated, begins summing adjacent final peak areas and heights until canceled by INTG() 14. All peaks whose retention times are between the two events are included; there are no partial peaks.

Summing occurs AFTER baseline construction, so the other integration functions may be used to control baseline construction. AR REJ can be used to eliminate small peaks from the sum.

The sum is reported as a single "peak" whose retention time is the average of the times for the start and the end of the Window.

If multiple sums are created, separate them by at least 0.02 minute if Peak Width is 0.16 or larger, and by 0.06 minute if it is 0.64 or larger.

If a new Window is started (using INTG() 14) while an earlier Window is still in effect, the HP 3396 terminates the first sum and then begins a new one.



5

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Curve Fits 5−19
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Understanding Calibrations

Understanding Calibrations

Introduction

This section first explains the calculation procedures used in calibrations, single and multi-level calibration, and peak identification, then gives detailed instructions for preparing different kinds of calibrations. It is designed for those analysts who desire a detailed explanation of the calibration process. If you are familiar with the use of calibration, you can proceed to Chapter 6, *Preparing a Calibration*. Chapter 6 explains how to set up a calibration using the HP 3396.

Calculation Procedures

The HP 3396 allows you to report the results of an integration from a real-time run or a reanalysis by using one of five calculation formulas. Two—the Area% and Height% formulas—do not require a calibration; hence they are uncalibrated procedures. The other three formulas require that a valid calibration table exist in the active workspace, and therefore are calibrated formulas. They are Normalization, External Standard, and Internal Standard.

What is a Calibration?

A calibration involves two steps. First, a standard with known amounts of the analytes of interest is prepared and analyzed. Then the samples, with unknown amounts of the same analytes, are analyzed under the same conditions. The detector response to the standards is used to help compute the actual amount of sample present. Several terms that are used in this chapter are defined below.

Measured Response, or Area:

The area of a peak relative to the chromatographic baseline. This is the final value produced by the integration software. See *Understanding Integration* for more information on how this value is determined. The area is a function of the amount of the peak components in the sample, the sample injection size, the amount of the material in the peak that elutes from the column, and the response of the detector to the peak components. The measured response is usually an area under a peak. The height of the peak can be used instead of the area; if it is, all calibrations will be based on height.

Corrected Response:

The result of applying a calibration to a peak area. This process corrects for the difference in sensitivity of the detector to different sample components and for retention of a peak component on the column. The HP 3396 calculates the corrected response from the area measured during a run and the sample amount supplied by the chromatographer. Obtaining a corrected response is the essence of calibration.

Multiplication Factor:

A parameter that corrects for dilutions, unit conversions, etc. If the sample contains the same amount of all components, and the multiplication factor is 1.00, the final amount of each component that the HP 3396 reports is in the same concentration units as the sample components. This can result in very small numbers. To change a reported result such as 0.000025 mg/ml to 25 ng/ml, enter a multiplication factor of 100,000. The default value is 1.00.

Reference Peak:

The HP 3396 identifies peaks by comparing their actual retention time to the retention times in the calibration run. Since times can vary somewhat from run to run, peak identification begins by locating reference peaks designated by the analyst. If the retention time has shifted for the reference peaks, the integrator assumes a similar shift has occurred for all peaks, and detects them accordingly. It is advisable to designate several reference peaks, near the beginning, middle, and end of the run. The reference peak can be any peak; it is calibrated and otherwise treated like a regular sample peak by the integrator. The reference peak must be the largest peak in the immediate vicinity, since if retention times shift and two or more peaks appear in the retention time window for the reference peak, the largest one is assumed by the integrator to be the reference peak.

Response Factor:

The response factor is determined by the following equation:

$$\text{Response Factor} = \frac{\text{(the known amount of material)}}{\text{(Area from a calibration peak)}}$$

It can also be described as the reciprocal of the slope of the curve of the area versus known amount. The response factor is determined by the HP 3396 from the measured area and the sample amount entered by the chromatographer. It is used to find the corrected response.

Corrected Response = (Area of sample peak) x (Response Factor)

The primary determinant of the response factor is the detector sensitivity to the sample component in question.

Sample Amount:

The sample amount is the weight or concentration of the original sample. It must either be in the same units or be corrected with the multiplication factor each time it is entered.

AREA PERCENT (AREA%)

Area% calculates the percentage of the total peak area represented by an individual peak. The formula used for Area% is:

$$(\text{Area\% of y}) = \frac{(\text{Area of y}) \times 100}{(\text{Sum of Areas of all peaks})}$$

Area% is the default calculation formula. It gives a good approximation of the relative amounts of material in a mixture if the detector is equally sensitive to all components and if all components elute completely from the column. Area% is used routinely when qualitative results are desired. It is also the report generated as the first step in preparing a calibration.

HEIGHT PERCENT (HEIGHT%)

The Height% formula reports the percentage of a detected component's height with respect to the total height of all components. It is the same formula as that used for Area%, with peak height used instead of area. Height% instead of Area% can be selected for reporting results in OP #4, and in the [PREP] [METH] and [EDIT] [METH] dialogs.

The other calibration procedures (normalization, ESTD, and ISTD) will all be calculated based on height instead of area if a HEIGHT% report is selected.

NORMALIZATION (NORM)

The normalization procedure is essentially the same as Area%, except that corrected responses instead of measured responses (or peak areas) are used in the calculation. This corrects for differences in detector sensitivity to different components of the sample.

The formula for normalization is:

$$(\text{percentage of y}) = \frac{(\text{corrected response of y}) \times 100}{(\text{Sum of corrected responses for all peaks})}$$

Normalization can provide better quantitative results than Area% and Height% because the measured areas are corrected for variations in detector response to the different components. However, two disadvantages of this method are that all components must be calibrated, even if only one is of interest; and the presence of components unseen by the detector, such as water with an FID, can greatly distort the calculations.

Since normalization is a relative value (%), the amount entered in the dialog has no effect.

Normalization is selected during [PREP] [CALIB] or in the [EDIT] [CALIB] dialog.

EXTERNAL STANDARD (ESTD AND ESTD%)

The External Standard formula reports the amount of each component in a sample with respect to the absolute response factors for the components. The multiplication factor must be entered to get an absolute amount. The equation for determining the ESTD amount is:

(amount of y) = (corrected response of y) x (MF)

MF is the multiplication factor. Remember that the equation for the corrected response of y is:

(Corrected Response) = (Area of sample peak) x (Response Factor)

A sample amount can be entered by the user in the [PREP] [CALIB], [OP()] 3, or [OP()] 7 dialogs, and in the sample information table. If a non-zero amount is entered, the ESTD% is reported instead of an absolute amount. The absolute ESTD amount, calculated above, and the sample amount are used to calculate the ESTD%. The equation for determining the ESTD% is:

$$(\text{ESTD\% of y}) = \frac{(\text{amount of y}) \times 100}{(\text{sample amount})}$$

The external standard amount can be reported in all units, and does not require calibration of peaks that are not of interest. However, ESTD is very sensitive to sample size; thus it is most suitable for use with an automatic sampler.

ESTD is selected during the [PREP] [CALIB] and [EDIT] [CALIB] dialogs.

INTERNAL STANDARD (ISTD AND ISTD%)

An internal standard is a component that is added to the standards that will be used in the calibration and to the samples to be analyzed. Since the same amount is present in every analysis, it serves as a reference or normalizing factor. The amount of the components of interest is reported relative to the internal standard. A properly chosen and precisely measured internal standard can provide the most reliable chromatographic data for quantitation.

The formula for the calculation of the amount of component y in a mixture relative to the internal standard is:

$$(\text{amount of y}) = \frac{(\text{corrected response of y}) \times (\text{amount of ISTD}) \times (\text{MF})}{(\text{corrected response of ISTD})}$$

If desired, a multiplication factor can be entered through [OP()] 7 or a sample table in [PREP] [CALIB]; if it is, the ISTD amount calculated in the equation above is multiplied by the multiplication factor.

If a non-zero sample amount is entered by the user in the [PREP] [CALIB], [OP()] 3, or [OP()] 7 dialogs, the ISTD% is calculated by the HP 3396 from the sample amount and the ISTD amount of y, calculated above.

In internal standard calculations, the AMOUNT is the total amount of sample to which the internal standard was added, but not including the internal standard.

The formula for internal standard is:

$$(\text{ISTD\% of y}) = \frac{(\text{ISTD amount of y}) \times 100}{(\text{sample amount})}$$

Remember that ISTD requires that an extra component, the internal standard, be added in a known amount to every sample. The retention time of this extra component must be different from the other components in the sample and the peaks must not overlap. This requirement is generally met easily with liquid samples but is impractical with gas samples. Fortunately, the availability of gas sampling valves circumvents the problem of reproducible sample size with gases and allows the External Standard method to be used effectively.

Samples and standards are analyzed and integrated using the same acquisition and integration parameters. However, in the internal standard method, the extra component (the internal standard) is treated differently in the generation of the reports. The advantages of using an ISTD are that sample size is not critical; instrument setpoint, flow, column drift, etc., are compensated for by the internal standard; only the peaks of interest need be eluted and measured; and each calibrated peak is calculated independently.

The major disadvantage of the ISTD calculation is that the internal standard must be added to every sample.

Selecting an Internal Standard

Some of the requirements for an internal standard compound are:

- It must be available in high (or at least known) purity.
- It must be stable, both on the shelf and during analysis.
- It must not react with any of the sample components.
- It must chromatograph well, yielding a well-formed peak.
- It must have a retention time different from any of the sample components.
- It must be soluble in the sample or in the solute used for the sample.
- It must elute near the components of interest.

Must the standard be chemically similar to the sample component? That depends on how it will be used. If it is added to the sample just prior to injection, with no intervening sample preparation steps, the answer is usually no. It is only important for it to chromatograph well under the same conditions as the sample.

You may want the standard peak and the unknown peaks to behave similarly in the detector so that they have similar linear ranges and response factors. For example, it would be inappropriate to use an internal standard which contains chlorine or phosphorous atoms (which lead to non-linear behavior in flame ionization detectors) when analyzing hydrocarbons.

If the internal standard is being added to a raw sample such as serum or plasma which is to be subjected to a series of extractions, concentrations, derivatizations and other steps before chromatography, the answer is yes. This is because there will be some component loss during this workup (recovery is never 100%) and you want the amount of internal standard to

have about the same ratio to the component after the workup as before. This is the situation in a great number of biochemical analyses.

If you do not have a preliminary workup problem, you should use the simplest possible compounds, since they need not resemble (chemically) the peaks you are analyzing. The normal hydrocarbons are a good choice in organic systems, and the primary alcohols are good for aqueous systems. The more complex the chemical is, the more likely you are to run into problems.

CONTENT OF THE CALIBRATION

The type and amount of information required for creating a calibration varies with the type of calculation procedure desired. Table 5-1 summarizes the information required for each calculation formula.

Table 1–1 Information Needed for Calibrations

	NORM	ESTD	ISTD
Calibration Table based on peak heights? (a)	OPT	OPT	OPT
One or more additional peaks may be included as non-reference peaks (b)	OPT	OPT	OPT
Retention time windows must be specified?	YES	YES	YES
One peak MUST be designated as an internal standard?	---	---	YES
Amount of internal standard in sample may be different from amount in calibration mixture?	---	---	YES
Sample amount must be specified?	NO	OPT	OPT

a. If the Calibration Table is created based on peak height, post-run calculations and reports are also based on peak height.

b. HP 3396 uses Calibration Table peak whose “CAL #” = 1 as a default reference, if none is entered by the user.

Calibration

The HP 3396 allows you to perform single-level or multi-level calibration. For multi-level calibrations, three calibration curve fits are possible: point-to-point, linear, and quadratic.

Choosing Single or Multi-level Calibration

Single level calibrations are used when the sample components exhibit a linear response to the detector that passes through the origin when graphed. A single point can be used because the response factor at every point is the same.

If the response is linear but doesn't pass through the origin, sample is probably being retained on the column. A multi-level calibration must be used to obtain the slope of the line. Usually only two levels are needed.

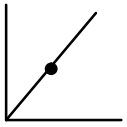
If the detector response to the sample components is non-linear, multi-level calibration must be chosen, and several levels must be used. However, non-linearity is not always the fault of the detector. An overheated injector can decompose the components; reactive or adsorbing column materials may remove material; and columns, detectors, and electronics may overload causing non-linearity. You may be able to correct these problems and avoid multi-level calibration. Multi-level calibration is something you do only when necessary. It is complicated, more time consuming than a single point calibration and needs to be checked more frequently, but it can handle situations that a single point calibration cannot.

Single level Calibration

Single level calibration primarily corrects for detector response differences between components present in a sample.

Single level calibrations have only one calibration entry per peak. The single-level (single-point) calibration assumes that:

- the response factor is independent of the amount of material in a peak (i.e., that response factor is a constant);
- the response (area or height) versus amount line passes through the origin (for instance, no sample is lost on the column).



Single level calibration

In general, single level calibration is useful for a linear response which passes through the origin. It can be described by a single number (the slope, or its reciprocal which is the response factor) that can be applied to all response data. The response curve is a straight line through the origin.

Outline of the Calibration Process

Preparing and using a single-level calibration consists of the following steps:

1. Prepare a calibration mixture in which the exact amounts of the components of interest are known. This calibration mixture should be prepared and analyzed using the same instrument conditions as those intended for subsequent sample runs.
2. Inject the calibration mixture and obtain an Area % or Height % report. After the run, the HP 3396 stores the peak retention times, peak areas, and peak heights in a processed peak file in the active workspace.

   Because peak information is stored for only one analysis at a time, preparation of the calibration table must follow analysis of the calibration mixture. If you do not plan to prepare the calibration immediately, be sure to save the data. See *Saving Integrator Data* for instructions.
3. Go to Chapter 6, *Preparing a Calibration*, for details of the calibration dialog.
4. Before you run your samples, edit the method to be used to include a calibration. Select the calibration file name if the calibration has been stored, or just leave the calibration in the active workspace. The HP 3396 will use the calibration in the active workspace to generate the sample report if no file name is indicated. After you prepare the calibration, store the method and the calibration will be saved as part of the method.

The Content of the Multi-level Calibration

Multi-level calibrations must always begin at the first level since this level must contain the retention times of any peaks calibrated in the first level and all subsequent levels. The type and amount of information required for creating a calibration varies with the type of calculation procedure desired. Refer to *Single Level Calibration* for a checklist of information required for a single level calibration.

Preparing a Multi-level Calibration

Follow the steps below to prepare a multi-level calibration.

1. Prepare a series of dilutions at known concentrations of the calibration mixture.
2. Prepare a single level calibration with the most dilute concentration. See *Preparing a Single Level Calibration* in Chapter 6.
3. Prepare additional levels for each dilution by following the detailed description of the dialog for *Preparing a Multi-level Calibration* in Chapter 6.
4. Store the calibration with a file name and load it before analyzing the samples, or leave the calibration in the active workspace and store the method.

After Multi-level Calibration

After the last level is created, the HP 3396 derives an equation that best fits the detector's response versus component amounts over all levels. This equation produces the response factor used to convert the measured areas (or heights) in sample runs into amounts or concentrations. An absolute response factor is computed for NORM and ESTD procedures. ISTD procedures produce relative response factors.

In subsequent sample runs, the integrator uses the computed response factor in the chosen calculation formula to report the amount, concentration, or percentage of each component in the sample.

Curve Fits

Multi-level calibration allows one of three curve fits: point-to-point, linear, and non-linear or quadratic. Each of these curve fitting procedures is described below. The best way to decide which curve fit to use for your analysis is to use the Applications Program *Plotting Calibration Curves.* In this Applications Program, the calibration is prepared with at least three levels. Then each curve fit can be selected and plotted with a few simple steps, and the graphs are examined for the best fit. The fit of each curve is calculated and expressed mathematically by the HP 3396 to help you select which one is most appropriate. See *Using Application Programs* for more details.

The default curve fit (point-to-point) can be used when you first prepare a multi-level calibration. Remember to save your data so that you can change the curve fit in a reanalysis if the fit does not seem appropriate. You can start by preparing a calibration with three levels. If a good curve fit is not obtained, you can add levels to the calibration.

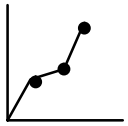
Point-to-Point Curve Fit

For each component in a multi-level calibration, the point-to-point curve fit computes a response factor for a detector's response that is higher than second order.

The point-to-point fit is often used to match an arbitrary response curve within the range of the identified calibration amounts. The segment of the response below the lowest calibrated amount is assumed by the HP 3396 to pass through the origin. Therefore, it is good practice to calibrate at a point below the lowest expected amounts for any samples whose response curves do not pass through the origin or are unknown.

The segment above the highest calibrated amount is assumed by the integrator to be a linear extrapolation of the segment between the second highest and the highest calibrated amounts. If the actual response above the highest level is unknown or does not follow this behavior, make sure that the highest calibrated amount is greater than any expected sample amounts.

An example of the point-to-point fit is shown below.



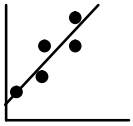
Point-to-point

Linear Curve Fit

The HP 3396's linear curve fit is also known as the least squares fit or linear regression technique. It may be used whenever the detector response is approximately linear and when the assumption of a linear response does not create a significant error. The linear fit may also be used for a narrow concentration range over which a somewhat linear detector response exists or can be assumed without producing substantial error, even though the curve as a whole is nonlinear. The calculation does not assume the plotted curve passes through the origin.

This curve fit computes a relative or absolute response factor for each calibrated component in a multi-level calibration. The integrator constructs a straight-line equation that best fits the response versus amount data points over all the levels in the multi-level calibration. The response factor (amt/response) is then used in conjunction with the selected calculation formula (normalization, ESTD, or ISTD) to report the amount, concentration, or percentage of each component in the sample.

The straight-line equation computes the response factor from the general straight-line equation in slope-intercept form. An example of a linear curve fit is shown below.

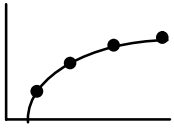


Linear

Non-Linear, Quadratic Curve Fit

The non-linear curve fit produces a response factor from a quadratic equation that best fits a detector's second-order response. It is desirable to have numerous calibration points when using this fit, since the shape of the parabola will fit the actual curve better when there are more points for the HP 3396 to use in constructing the curve.

The curve-fitting technique uses a least squares method to fit the the detector's response. The general quadratic equation is used to compute the response factor for each component in a multi-level calculation. An example of a quadratic curve fit is shown below.



Non-linear (quadratic)

Examples

In this section, several examples of calibration will be demonstrated. You can just read over the examples, or make a real chromatographic run with any sample and treat the data obtained in the same way the example data is treated. If you use your real sample for these examples, you may be able to decide whether single level calibration is sufficient for your analyses, or whether you must use multi-level calibration.

1. Prepare a Method

   A detailed explanation of the method preparation dialog is found in Chapter 8, *Using Methods*. If you obtain an AREA% report, your calibration will use peak areas. If you obtain a HEIGHT% report, your calibration will use peak heights.

   Don't forget to save the data obtained from your chromatogram so you can reanalyze the same data to see the effect of changing calibration parameters.

2. Prepare the Sample

   If you are using your own sample, prepare a solution at any known reasonable concentration for a single level calibration. Prepare the most dilute sample for the first level of a multi-level calibration.

3. Obtain a Chromatogram and Report

   Unless you selected a HEIGHT% report, the report will be in AREA%, which is the default reporting option. The retention times obtained on this report will be used to identify the peaks in the calibration table. The measured areas (or heights) will be used to calculate the reponse factors. If you are analyzing a real sample, inject and press START.

Note: Be sure to elect to save your signal data in the method preparation dialog.

4. Press [PREP] [CALIB] [ENTER]

   Each question in the dialog that follows is explained in detail in Chapter 6, *Preparing a Calibration*. If you just want to see the effect of changing various parameters on the report, follow the examples below. You may want to read over Chapter 6 first to learn what the dialog prompts mean. If you are preparing a real calibration, use the data you obtained with the actual component amounts in your sample. In these examples, your response to the dialog prompts is shown in bold print. After every input, press [ENTER].

Example 1. Normalization

You work in a quality control lab and perform routine analyses to verify that the correct components are present in the raw chemicals you purchase. You seldom use an autosampler for these analyses, because only one or two samples are analyzed on an irregular basis, just before the chemical is used in the production plant. What you are interested in is the ratio of the sample components to each other, and all sample components are important. You use **normalization** to calibrate with the standard chemical before analyzing a new shipment. The following report was obtained from the sample injected.

```
RUN #10        July 5, 1994   08:47:06

METHOD NAME:            M:NORM.MET

SIGNAL FILE:            L:Q8A4A08E.BNC

AREA %

 RT        AREA      TYPE      WIDTH     AREA%
 .250      316468    BB        .134      18.3737
 .750      126610    BB        .134       7.3508
1.250      12601     BB        .134      0.7316
1.750      1266714   PB        .134      73.5438

TOTAL AREA=1722393

MUL FACTOR=1.0000+E00
```

Now start the calibration preparation dialog.

***[PREP] [CALIB] [ENTER]**

If there is a calibration already present in the active workspace the integrator will print CALIB EXITS. If you get this message, press the function keys [DEL] [CALIB] [ENTER] to delete the calibration, then press [PREP] [CALIB] [ENTER] again. Only one calibration can exist in the active workspace.

```
E = EXTERNAL STANDARD

I = INTERNAL STANDARD

N = NORMALIZATION

CALIB PROCEDURE [E*/I/N]        N [ENTER]

REF % RTW [5.000]          [ENTER]

NON-REF % RTW [5.000]      [ENTER]

RF BASED ON AREA OR HEIGHT [A*/H]    [ENTER]
```

CAL #	RT	AMT	NAME*
1	**0.250 [ENTER]**	**1 [ENTER]**	**[ENTER]**
2	**0.750 [ENTER]**	**1 [ENTER]**	**[ENTER]**
3	**1.250 [ENTER]**	**1 [ENTER]**	**[ENTER]**
4	**1.750 [ENTER]**	**1 [ENTER]**	**[ENTER]**
5	**[ENTER]**		

*The sample names can be left blank; just press [ENTER].

The calibration table is prepared by filling in a calibration number, retention time, amount, and name, if desired, for each peak. The retention time must exactly match the time of a peak in the run in current memory to be valid. If you fill in a retention time that is not an exact match, the integrator will print NO MATCH and prompt with the same calibration number again. In a normalization calibration, all the peaks must be included. When all the peaks are listed, press [ENTER] to terminate this part of the dialog.

A reference peak can be designated by putting a minus sign before the retention time of the reference peak in the table above. If one is not designated, the HP 3396 prompts for a reference peak.

`REF PK CAL #:` **1 [ENTER]**

`REF PK CAL #:` **4 [ENTER]**

`REF PK CAL #:` **[ENTER]**

The calibration number of the peak to be used as a reference peak is entered, or peak number 1 is used if no peak is selected. After filling in as many reference peaks as desired, press [ENTER] to move on to the next prompt.

`GROUP PEAKS [Y/N*]:` **[ENTER]**

Isomers or other similar peaks can be grouped together. For this exercise, choose N.

`RF of Uncalibrated Peaks [0.0000E+00]:` **[ENTER]**

`Replace calibration fit [Y/N*]:` **[ENTER]**

The choice of fit is irrelevant for a single level calibration.

`Disable post-run RT update [Y/N*]:` **[ENTER]**

See Chapter 6, *Preparing a Calibration,* for an explanation of these prompts.

`SAMPLE AMT [0.0000E+00]:` **[ENTER]**

The sample amount is not used in a normalization calibration; because the value obtained is expressed as a percentage of the total components. Just press [ENTER] to go on.

`MUL FACTOR [1.0000E+00]:` **[ENTER]**

The multiplication factor is irrelevant; we are only interested in the relative amounts of components.

The calibration preparation dialog ends with the multiplication factor. At this point, you can press [LIST] [CALIB] to review your calibration, or [REPORT] to see how the report looks with the calibration included. If you think you may want to use your calibration again, you should store it with an appropriate file name so you don't accidently overwrite it. Now when you start a new run or a reanalysis, the calibration will be used in the report. If you are doing an analysis, inject your sample and press [START]. The chromatogram and the normalization report from this analysis is shown below.

```
RUN #139        July 5, 1994   08:12:06

METHOD NAME:              M:SAVEL.MET

SIGNAL FILE:              L:Q8A4A857.BNC

NORM-AREA

 RT         AREA       TYPE       CAL#       AMOUNT

 .249       316456     BB         1R         25.005

 .749       31630      BB         2          25.008

 1.250      3139       BB         3R         24.997

 1.749      316668     PB         4          24.899

TOTAL AREA=172293

MUL FACTOR=1.0000+E00
```

We have four components that are each present at an equal level, so each is 25% of the total. The response factors correct the variations in measured area to obtain the correct percentage.

Example 2. External Standard (ESTD)

You work for a medium sized lab that has to perform several hundred EPA analyses per month on their own wastewater to monitor three carcinogens. Since you have so many identical analyses, you use an autosampler. The report you fill in for the EPA must give the absolute amounts of the hazardous components present in the wastewater; therefore you select an external standard calibration. You do a purge-and-trap concentration of 5 ml of water for each analyses. You know that the calibration curve is not linear for the three components of interest, so you prepare a point-to-point multilevel calibration. You expect your samples to have less than 75 ppb of each contaminant. If they contain less than 20 ppb of any analyte, the concentration is considered below the limit of quantitation. For your standards, you prepare dilutions of the components of interest at 20 ppb, 40 ppb, and 80 ppb.

Follow steps 1 through 4 near the beginning of this section to get ready to prepare the calibration. The first step in a multi-level calibration is to prepare a single level calibration. A single level ESTD calibration is demonstrated on the following pages. After preparing the single level calibration, you repeat the process by pressing [PREP] [CALIB] (level number) [ENTER] to prepare the next level of the calibration. See Chapter 6 for a detailed explanation of the dialog prompts.

First do a run with the amount of sample that will be used for the first calibration level. You will get a report similar to that shown below.

```
RUN #10         July 5, 1994    08:47:06

METHOD NAME:              M:ESTD.MET

SIGNAL FILE:              L:Q8A4A08E.BNC

AREA %

 RT         AREA       TYPE       WIDTH      AREA%
 .250       316468     BB         .134       47.3817
 .750       126610     BB         .134        5.2356
 1.750      316472     PB         .134       47.4222
TOTAL AREA=759550

MUL FACTOR=1.0000E+00
```

The HP 3396 will calculate the response factors for the first level based on the areas detected here, and the amount entered in the first level calibration dialog demonstrated below.

***[PREP] [CALIB] [ENTER]**

```
E = EXTERNAL STANDARD

I = INTERNAL STANDARD

N = NORMALIZATION

CALIB PROCEDURE [E*/I/N]        [ENTER]

REF % RTW [5.000]          [ENTER]

NON-REF % RTW [5.000]      [ENTER]

RF BASED ON AREA OR HEIGHT [A*/H]     [ENTER]
```

CAL #	RT	AMT*	NAME**
1	**0.250 [ENTER]**	**100 [ENTER]**	**xxx [ENTER]**
2	**0.750 [ENTER]**	**100 [ENTER]**	**yyy [ENTER]**
3	**-1.750 [ENTER]**	**100 [ENTER]**	**xyz [ENTER]**
4	**[ENTER]**		

*Each standard is present at a level of 100 ng/5 ml of water, or 20 ppb.

**The sample names can be left blank; just press [ENTER].

The calibration table is prepared, filling in a calibration number, retention time, amount, and name for each peak. The retention time must exactly match the time of a peak in a run in current memory to be valid. If you fill in a retention time that is not an exact match, the integrator will print NO MATCH and prompt with the same calibration number again. You don't have to include all the peaks for an ESTD calibration; just skip any that you don't want to calibrate. However, the first level must be filled in for any peak that you want to calibrate at any level. When all the peaks to be calibrated are listed, press [ENTER] to terminate this part of the dialog.

A reference peak is designated by putting a minus sign before the retention time of the reference peak. If one is not designated, the HP 3396 prompts for a reference peak.

`GROUP PEAKS [Y/N*]:` **[ENTER]**

Since we want to obtain results for individual peaks, we do not want to group any peaks.

`RF of Uncalibrated Peaks [0.0000E+00]` **[ENTER]**

`Replace calibration fit [Y/N*]:` **Y [ENTER]**

This is where a calibration curve fit is selected for a multi-level calibration.

```
P = point-to-point

L = linear

N = non-linear (quadratic)

Calibration fit [N/L/P*]:
```
[ENTER]

We will leave the curve fit at the default (point-to-point) until we see the results.

```
Disable post-run RT update [Y/N*]:
```
[ENTER]

See Chapter 6, *Preparing a Calibration,* for an explanation of these prompts.

```
SAMPLE AMT [0.0000E+00]:
```
[ENTER]

If a sample amount is entered, the results from the calibration will be in ESTD%. For an amount expressed as an absolute result, leave this empty.

```
ISTD AMT
```
[ENTER]

Since we aren't doing an ISTD calibration, just press [ENTER]. The HP 3396 will ignore the ISTD anyway.

```
MUL FACTOR [1.000E+00]:
```
[ENTER]

There is no multiplication factor involved in this analysis.

The calibration preparation dialog ends with MUL FACTOR.

You can obtain a report for the previous run that reflects the calibration information you just entered by pressing [SHIFT] [REPORT]. The report follows.

```
RUN #10        July 5, 1994   08:47:06

METHOD NAME:            M:ESTD.MET

SIGNAL FILE:            L:Q8A4A68E.BNC

ESTD - AREA

 RT        AREA          TYPE      CAL #      NG*
 .250      316468        BB        1          100.063
 .750      126610        BB        2          100.009
 1.750     316472        PB        3R          99.704
TOTAL AREA=759550

MUL FACTOR=1.0000E+00
```

Now do a run with the amount of material you want in the next calibration level. In this example, we will do a purge and trap with 40 ppb, or 200 ng, of each sample. The report obtained is shown on the following pages.

```
RUN #11        July 5, 1994   09:47:06

METHOD NAME:            M:ESTD.MET

SIGNAL FILE:            L:Q8A4A68E.BNC

ESTD - AREA

 RT        AREA          TYPE      CAL #      NG*

 .250      696229        BB        1          200.027

 .750      227898        BB        2          199.989

 1.750     632944        PB        3R         200.005

TOTAL AREA=1557071

MUL FACTOR=1.0000E+00
```

*The label for this column is selected in the REPORT section of the method preparation dialog. The default label is AMOUNT.

Press **[PREP] [CALIB] 2 [ENTER]** to enter the amounts for the next calibration level. The integrator prompts with the calibration level and retention time, and you fill in the amount.

CAL #	RT	AMT
1	0.250	**200 [ENTER]**
2	0.750	**200 [ENTER]**
3	1.750	**200 [ENTER]**

The second level calibration is now finished. At this point, if you press the [LIST] [CALIB] function keys you get the report below.

```
ESTD

REF % RTW:      5.000    NON-REF % RTW:          5.000

LEVEL:          1        RECALIBRATIONS:         1

LEVEL:          2        RECALIBRATIONS:         1

 CAL #     RT          LV        AMT             AMT/AREA
 1R        0.250       1         1.0000E+02      3.1599E-05
                       2         2.0000E+02      1.579E-05
 2         1.250       1         1.0000E+02      3.1827E-03
                       2         2.0000E+02      1.5872E-03
 3         1.750       1         1.0000E+02      3.1599E-05
                       2         2.0000E+02      1.5872E-05
```

The sample is now run at the next concentration, which in this example is 80 ppb or 400 ng. The report generated follows.

```
RUN #12        July 5, 1994   10:18:00

METHOD NAME:            M:estd.MET

SIGNAL FILE:            L:Q8B3939E.BNC

ESTD-AREA

 RT          AREA        TYPE       CAL#      NG

 .249        322835      BB         1R        400.003

 0.749       445796      BB         2         400.065

 1.750       2034402     BB         3         399.998

TOTAL AREA=2034402

MUL FACTOR=1.0000E+00
```

These results will be used for calibration level 3. You can proceed to add additional calibration levels. For each level, prepare a different concentration of the components of interest, inject the sample (or, as in this example, do a purge-and-trap of the sample), and obtain a chromatogram. Then press [PREP] [CALIB] (level number) [ENTER] to start the dialog for that level of the calibration curve.

[PREP] [CALIB] [3] [ENTER]

The **3** is for the third level. This time we have a 80 ppb sample in 5 ml water, or 400 ng.

CAL #	RT	AMT
1	.249	**400 [ENTER]**
2	.749	**400 [ENTER]**
3	1.749	**400 [ENTER]**

We now have a three level point-to-point calibration curve.

It is best to save the calibration with an appropriate file name at this point. See *Storing Calibrations*, Chapter 6. Also at this point, you could use the Application Program "Plotting Calibration Curves", in *Using Application Programs*, to be sure you are happy with the curve for each analyte, before running the samples.

The final step is to load up the autosampler with your samples and run them. A series of autosampler runs is usually run as a sequence. Sequence preparation is explained in *Automating Analysis*, Chapter 9. The report for each chromatogram in the sequence will be an ESTD report, based on the calibration curve prepared here.

Example 3. Internal Standard (ISTD)

You work in a pharmaceutical laboratory that is testing a possible miracle cure for cancer. Your routine analyses include testing blood samples from the rats who have received the drug to see how long the drug lasts before it is metabolized. After the blood samples are sent to your lab, you perform a series of extractions, centrifugations, etc., to prepare the blood for analysis by liquid chromatography. You decide to use an internal standard for calibration. The internal standard will be added to the blood samples before extraction.

The first step in the extraction of the blood samples is to mix each one with an equal amount of chloroform, so you decide to spike the chloroform with 50 ug/ml of the internal standard before mixing it with the blood samples. You prepare enough chloroform with internal standard for both the standard runs and all the blood samples, to be sure that each sample will have an identical amount of internal standard. To prepare the standards, you spike four blood samples from rats that have never been given the miracle drug with different drug levels. You decide that using levels of 10, 50, 100, and 200 ug/ml will bracket all expected concentrations of drugs in the blood samples. You extract the blood samples with standard in the same way as the samples are treated.

After following the extraction procedure for the blood samples, you prepare the ISTD multi-level calibration curve. Follow steps 1 through 4 at the beginning of this section, using the lowest level of standard to obtain the chromatogram for the first calibration level, then start the calibration dialog.

```
RUN #1          July 25, 1994  08:07:06
METHOD NAME:              M:IST.MET
SIGNAL FILE:              L:Q8A4A19E.BNC
AREA %

 RT         AREA      TYPE      WIDTH     AREA%
 .250       225422    BB        .134      13.9755
 .750       120610    BB        .134      7.4785
 1.750      1266714   PB        .134      78.5439
TOTAL AREA:=1612746
MUL FACTOR=1.00E+00
```

***[PREP] [CALIB]**

```
E = EXTERNAL STANDARD
I = INTERNAL STANDARD
N = NORMALIZATION
CALIB PROCEDURE [E*/I/N]        I [ENTER]
REF % RTW [5.000]               5 [ENTER]
NON-REF % RTW [5.000]           5 [ENTER]
RF BASED ON AREA OR HEIGHT [A*/H]   A [ENTER]
```

CAL #	RT	AMT	NAME
1	**0.249 [ENTER]**	**50 [ENTER]**	**INT STD [ENTER]**
4	**0.749 [ENTER]**	**10 [ENTER]**	**Miracle Drug [ENTER]**
7	**[ENTER]**		

The calibration table is prepared, filling in a calibration number, retention time, amount, and name if desired for each peak. The retention time must exactly match the time of a peak in a run in current memory to be valid. If you fill in a retention time that is not an exact match, the integrator will print NO MATCH and prompt with the same calibration number again. You don't have to include all the peaks for an ISTD calibration; we know from previous experiments that the drug elutes at 0.749 minutes. The other peaks are blood components that do not interest us.

A reference peak is designated by putting a minus sign before the retention time of the reference peak. If one is not designated, the HP 3396 prompts for a reference peak. It is a good idea to designate the internal standard as one of the reference peaks, since it was selected to be a large, well separated peak.

`REF PK CAL #:` **1 [ENTER]**

`REF PK CAL #:` **[ENTER]**

`ISTD CAL #:` **1[ENTER]**

`GROUP PEAKS [Y/N*]:` **[ENTER]**

`RF of Uncalibrated Peaks [0.0000E+00]:` **[ENTER]**

`Replace calibration fit [Y/N*]:` **Y [ENTER]**

This is where a calibration curve fit is selected for a multi-level calibration.

```
P = point-to-point

L = linear

N = non-linear (quadratic)
```

`Calibration fit [N/L/P*]:` **L [ENTER]**

We think the response in linear. The fit can be changed during reanalysis, so it is not necessary to know what fit to use.

`Disable post-run RT update [Y/N*]:` **N [ENTER]**

See Chapter 6, *Preparing a Calibration,* for an explanation of these prompts.

SAMPLE AMT [0.0000E+00]: **[ENTER]**

ISTD AMT **50 [ENTER]**

We want the internal standard to be at about the same peak size as the standard peaks. The internal standard responds the same in the UV detector as the drug, so we use an amount that is about the peak size of the standards, which will be 10-200 ug.

MUL FACTOR [1.000E+00]: **[ENTER]**

The calibration preparation dialog ends with MUL FACTOR.

The first level is prepared. If you press [SHIFT] [REPORT] you will obtain the report shown below.

```
Run # 1        Jul 25, 1994      08:07:06

METHOD NAME:            M:IST.MET

SIGNAL FILE:            L:Q8A419E.BNC

ISTD-AREA

 RT        AREA      TYPE      CAL #     AMOUNT

 .249      225890    BB        1&

 .749      482440    BB        2         10.004

TOTAL AREA=708330

MUL FACTOR=1.0000E+00

ISTD AMT=50.0000E+00
```

Now proceed to inject the sample for level 2. The report is shown below.

```
Run # 2         Jul 25, 1994       09:51:42

METHOD NAME:              M:IST.MET

SIGNAL FILE:              L:Q8A710BC

ISTD-AREA

 RT         AREA       TYPE       CAL #      AMOUNT

 .249       225271     BB         1&*

 .749       964880     BB         2          49.940

TOTAL AREA=1190151

MUL FACTOR=1.0000E+00

ISTD AMT=50.0000E+00
```

***Note:** The "&" after a peak indicates it is both the internal standard and a reference peak. If the peak was just the internal standard, it would have an "S".

Press **[PREP] [CALIB] 2 [ENTER]** to start the dialog for level 2 of the calibration curve.

***[PREP] [CALIB] [2] [ENTER]**

The 2 is for the second level. The prompts will ask for the new sample amount.

```
 CAL #        LEVEL        AMT

 1            2            50 [ENTER]
```

Prepare the rest of the calibration levels in the same way. In our drug analysis, we will use 100 ug at level 3 and 200 ug at level 4. This gives us a four level linear calibration curve, that surrounds the sample concentrations we expect. The last two reports are shown on the following pages.

```
Run # 4        Jul 25, 1994      12:45:42
METHOD NAME:            M:IST.MET
SIGNAL FILE:            L:Q8A608BC
ISTD-AREA

 RT        AREA        TYPE        CAL #       AMOUNT
 .249      225613      BB          1&
 .749      1929760     BB          2           100.004
TOTAL AREA=2155373
MUL FACTOR=1.0000E+00
ISTD AMT=50.0000E+00

Run # 5        Jul 25, 1994      13:01:42
METHOD NAME:            M:IST.MET
SIGNAL FILE:            L:Q8A608BC
ISTD-AREA

 RT        AREA      TYPE      CAL #     AMOUNT
 .249      225497    BB        1&
 .749      3859687   BB        2         200.124
TOTAL AREA=4085184
MUL FACTOR=1.0000E+00
ISTD AMT=50.0000E+00
```

It is best to save the calibration with an appropriate file name at this point. See *Storing Calibrations*, Chapter 6. Also at this point, you could use the Application Program “Plotting Calibration Curves”, in *Using*

Application Programs, to be sure you are happy with the linear curve fit, before running the samples.

The final step is to load up the autosampler with your samples and run them. A series of autosampler runs is usually run as a sequence. Sequence preparation is explained in *Using Sequences*, Chapter 9. The report for each chromatogram in the sequence will be an ISTD report, based on the calibration curve prepared here. An example of the report you might obtain from these analyses is shown below.

```
Run # 6         Jul 25, 1994       15:15:42

METHOD NAME:              M:IST.MET

SIGNAL FILE:              L:Q8A608BC

ISTD-AREA

 RT         AREA       TYPE       CAL #      AMOUNT

 .249       225012     BB         1&

 .749       252201     BB         2          19.034

TOTAL AREA=2798299

MUL FACTOR=1.0000E+00

ISTD AMT=50.0000E+00
```

6

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Using Calibrations

Using Calibrations

Introduction

This chapter explains the meaning of the prompts in the calibration preparation dialog, and gives detailed instructions for preparing a calibration.

If you have performed calibrated analyses before and understand the calibration process, this chapter will give you the information you need to use the HP 3396 to set up a calibration curve and analyze your samples. If calibration is new to you, you should read Chapter 5, *Understanding Calibrations*, before you proceed with this chapter. Chapter 5 explains the calibration calculations, how to select a calibration, when to use single and multi-level calibration, and the curve fits available.

Preparing a Single-Level Calibration

Preparing a single-level calibration (with some modifications) is also the first step in creating a multilevel calibration.

1. **Prepare and analyze a standard mixture containing known amounts of all the components to be calibrated.**

For the ISTD calculation, the mixture must contain the internal standard.

The HP 3396 uses the retention times and areas from this analysis and the standard amounts that will be entered by the user in the following dialog to calculate the response factors for the first calibration level.

Calibration Procedure

2. **Press [PREP] [CALIB] [ENTER] to begin the preparation dialog.**

If there is no processed peak data in the active workspace (it should be from the calibration mixture analysis), the HP 3396 prints `NO RUN PEAKS STORED`.

If a calibration already exists in the active workspace, this message appears `CALIB EXISTS` and the dialog terminates. Instructions for editing an existing calibration are located later in this chapter. To create a completely new calibration, you must delete the existing one. You may want to store it away as a file before deleting it from the workspace; see *Storing Calibrations* later in this chapter.

```
E = EXTERNAL STANDARD
I = INTERNAL STANDARD
N = NORMALIZATION

CALIB PROCEDURE (E*/I/N):
```

3. **Press [ENTER] to leave the selection unchanged or enter the appropriate letter for the desired calculation.**

The * after E indicates the currently-selected procedure (ESTD).

Retention Time Windows

```
REF % RTW [5.000]:
```

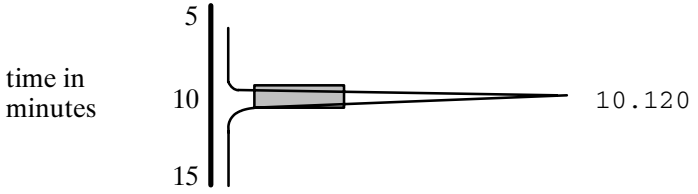
4. **Press [ENTER] to leave the selection unchanged or enter a new retention time window for reference peaks. Enter the value as**
{ **[−]** } *window half-width* **[ENTER].**

This entry sets a window in which the HP 3396 will search for reference peaks. If **[−]** is used, the half-width is an absolute number in minutes. When **[−]** is omitted, it is a percentage of the retention time of the reference peak being searched for. See *Retention Time Updating* at the end of this chapter for more information.

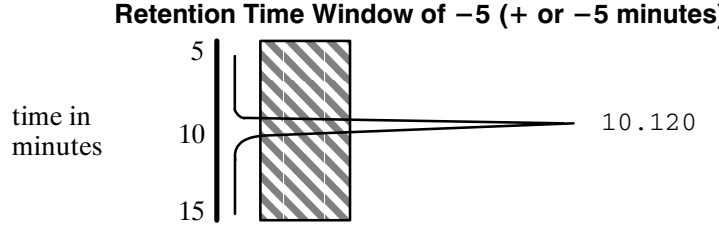
Example `REF % RTW [  5.000]:` **[ENTER]**

If the calibration retention time of a reference peak is 10 minutes and *window half-width* is 5, the search window extends from 9.5 minutes to 10.5 minutes (10 plus and minus 5%).

Retention Time Window of 5 (+ or −5%)



If the half-width was entered as −5, the window extends from 5 minutes to 15 minutes (10 plus and minus 5 minutes).




```
NON-REF % RTW [   5.000]:
```

5. **Press [ENTER] to leave the selection unchanged or enter a new retention time window for non-reference peaks. Enter the value as** { **[−]** } *window half-width* **[ENTER].**

```
RF BASED ON AREA OR HEIGHT [A*/H]:
```

6. **Press [ENTER] to base the calibration on peak area or enter [H] to base it on peak height.**

Table Entries

```
CAL#      RT          AMT          NAME
  1   :
```

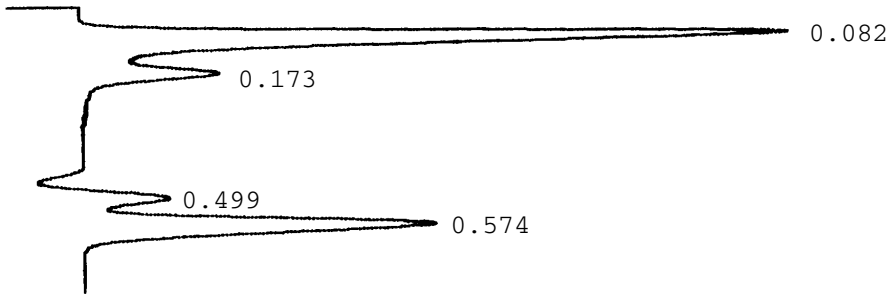
7. **Type the retention time to 3 decimal places and press [ENTER]. Type the amount and press [ENTER]. Typing the name (up to 16 characters) for each peak is optional. Press [ENTER] to proceed to the next line. Repeat this step for each peak to be calibrated. Press [ENTER] to terminate this part of the dialog.**

The retention time entered must be an exact match to the peak retention time in the report of analysis for the calibration mixture.

To designate a reference peak, enter the retention time, as above, but preceded by a minus sign. An entry of 5.20 designates an “ordinary” peak; an entry of −5.20 makes it a reference peak.

Example

```
CAL#      RT          AMT          NAME
  1   :-.082     :10    : S1       minus sign indicates reference peak
  2   : .173      :5    : S2
  3   : .498  NO MATCH   S3       retention times must match exactly
```



If you do not specify (with a **[−]** retention time entry) any reference peaks, the instrument will prompt you for it.

```
REF PK CAL#:
```

8. **Type the CAL# of a reference peak or press [ENTER] to use CAL #1 as the only reference peak.**

This prompt repeats allowing you to enter all reference peaks until you terminate by replying with **[ENTER]**. If the calculation procedure selected earlier is Internal Standard,

```
ISTD CAL#:
```

asks you to identify the internal standard peak. Responding with **[ENTER]** (no CAL#) selects CAL# 1.

Peak Groups

You may wish to have the sums of groups of peaks reported. This is useful when isomers of the same compound appear as separate peaks.

```
GROUP PEAKS [Y/N*]:
```

9. **Type Y to sum groups of peaks or type N to leave peaks ungrouped.**

If you do not choose to group peaks, the dialog continues with `CALIBRATION OPTIONS` and step 12.

When you choose to group peaks, calculated amounts for peaks will be summed in the groups that you specify in this dialog.

```
GRP#      CAL#                  NAME
1   :
```

10. **Type the desired CAL# and press [ENTER]. Press [ENTER] again when the group is complete. Type a name for the peak group.**

A group may contain up to 63 entries. A given CAL# may be entered more than once; it will be added into the group sum as many times as it appears. Each peak in a group retains its own calibration curve. Up to 127 groups can be entered. `NAME` is an optional 16 character group name.

Example

```
GRP#      CAL#              NAME
1    :7   ,9   ,10          :BUTENE
```

11. **Press [ENTER] when the GRP# is requested to terminate the grouping dialog.**

Do not use the **[BREAK]** key to interrupt the group process; the entire group in progress will be lost. Instead, finish the dialog normally and then reenter or use **[EDIT] [CALIB]** to correct the errors.

Calibration Options

```
CALIBRATION OPTIONS
RF of uncalibrated peaks [0.0000E+00 ]:
```

12. **Enter the response factor (Amount/Area or Amount/Height) to be used for uncalibrated peaks.**

For ISTD, enter a relative response factor (Response Factor for the uncalibrated peaks divided by the Response Factor for the internal standard peak).

```
Replace calibration fit [Y/N*]:
```

13. **Type N to leave the calibration fit unchanged.**

The choice of fit is irrelevant for a single level calibration. The fit is a straight line through the origin and the single calibration point.

```
Disable post-run RT update [Y/N*]:
```

14. **Type Y and press [ENTER] to disable retention time updating or type N to enable updating.**

Retention time updating updates retention times in the calibration table to reflect shifts observed in sample runs. See *Retention Time Updating* at the end of this chapter for more information.

```
ISTD AMT [0.0000E+00 ]:
SAMPLE AMT [0.0000E+00 ]:
MUL FACTOR [1.0000E+00 ]:
```

15. **Enter the Internal Standard amount (for ISTD calculations). Enter the sample amount (for ESTD% or ISTD% calculations). Enter the multiplication factor.**

For an ISTD calibration, you will be prompted for `ISTD AMT`, `SAMPLE AMT`, and `MUL FACTOR` at this time. For an ESTD calibration, you will be prompted for `SAMPLE AMT` and `MUL FACTOR`. For a NORM calibration,

you will only be prompted for `MUL FACTOR`. Enter the appropriate values, or press **[ENTER]** alone to skip to the next prompt and use the current value (displayed in brackets). The **[PREP] [CALIB]** dialog ends with the `MUL FACTOR` entry.

Preparing a Multilevel Calibration

When preparing a multilevel calibration, use the most dilute calibration mixture and create a single level calibration as described above, with the exception of steps 12 and 13. Follow the instructions for steps 12 and 13 below. After finishing the first level, add additional levels as described on the next page.

```
CALIBRATION OPTIONS

RF of uncalibrated peaks [0.0000E+00 ]:
```

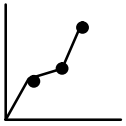
12. **Press [ENTER] to leave the response factor (Amount/Area or Amount/Height) at zero.**

```
Replace calibration fit [Y/N*]:
```

13. **Type Y and press [ENTER] to choose a calibration curve fit or press [ENTER] to keep the current calibration fit.**

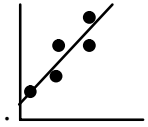
```
P = point-to-point
L = linear
N = non-linear (quadratic)
Calibration fit [N/L/P*]:
```

Use the descriptions below to select the desired fit. Note that a linear curve fit requires at least two calibration levels and a quadratic curve fit requires at least three. If you don't provide enough levels for the curve fit selected, the HP 3396 uses the default point-to-point fit.



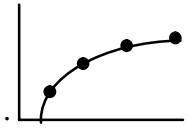
Point-to-point

The point-to-point fit will match any response curve within the range of the identified calibration amounts. The curve begins at the origin. It is good practice to calibrate at a point below the lowest expected amount for any component whose response curve does not pass through the origin or whose behavior is not known. The last segment (above the highest calibrated amount) is extended upward indefinitely.



Linear

This least squares fit requires at least two calibration levels to calculate the line. It does not necessarily pass through the origin as a single level calibration does.



Non-linear (quadratic)

This fit requires at least three calibration levels to calculate the curve, which is a parabola. The origin is not assumed.

See *Using Application Programs* for related information about plotting calibration curves.

Adding Additional Levels

1. **Make a calibration run with a calibration mixture appropriate for the new level. (The calibration must already exist.)**
2. **Press [PREP] [CALIB]** *level number* **[ENTER]**

level number is a number from 2 to 63 that has not been used already in the calibration file.

```
CAL#      RT          AMT

  1   :   rr.rrr    :
```

3. **Enter the amount of each component of interest in the particular level. Press [ENTER] after the AMT prompt to skip a peak.**

The CAL# and retention time (rr.rrr) prompts are from level 1. All peaks need not be present in all levels. New peaks (those not in level 1) must be edited in with the **[EDIT] [CALIB]** dialog. The new level is stored with existing levels in the active workspace.

4. **For ISTD calibrations, enter the ISTD amount for each level as well.**

Using Arbitrary Calibration Tables

If no Calibration table exists in the active workspace, one can be entered without first having to run a sample. To create an arbitrary calibration, first delete any run information in the active workspace, since the HP 3396 will try to use the current run data if any is available.

1. **Press [EDIT] [CALIB]** {*level number*} **[ENTER] to create an arbitrary calibration.**

level number is only required when you wish to edit an upper level of a multi-level calibration.

The dialog is the same as the **[PREP]** or **[EDIT] [CALIB]** dialog. You will be prompted for AMT/AREA (or AMT/HEIGHT) in addition to the other table parameters, because, since you are not using peak data, the HP 3396 does not calculate the response factors for you.

For all three calibration types, the HP 3396 expects an absolute response factor. For an ISTD calibration, the instrument will calculate the relative response factor for each component at each level from the absolute numbers provided for the component and the ISTD peak at that calibration level.

Using Recalibration

Calibrations have a limited lifetime because instrument sensitivity drifts and recalibration becomes necessary to maintain the accuracy of the analysis. Recalibration updates the Calibration Table either by averaging response data over a number of calibration runs or by replacing all response data.

For valid recalibration to occur, these conditions must be met:

- Peak information from a calibration run must be available in the active workspace.
- A calibration based on the same calibration mixture must exist.
- At least one reference peak must be found.
- For the Internal Standard procedure, the ISTD peak must be found.

1. **Press [CALIB]** *level number* **[ENTER] to manually initiate an averaging of response data.**

   or

   Press [CALIB] [−] *level number* **[ENTER] to replace the old response data with the new data rather than averaging them.**

Recalibrations may be automatically sequenced or can be manually initiated. The calculation uses a simple average of the data. An error message will be printed and the procedure aborted if any condition fails. Recalibration always uses amounts in the Calibration Table, regardless of where else they may be specified (e.g. the Sample Information Table). Recalibration can be ordered by a Sample Information Table entry to occur automatically during a series of runs.

Editing a Calibration

Calibrations may be edited using the **[EDIT] [CALIB]** or [**OP()] [3**] dialogs.

1. **Press [EDIT] [CALIB]** {*level number*} **[ENTER] to edit a calibration.**

level number is only required when you wish to edit an upper level of a multi-level calibration.

If you press **[EDIT] [CALIB] [ENTER]** when *no* calibration is present, the HP 3396 starts the dialog for preparing a new calibration without calibration run data. With no sample information in the active workspace (or ignoring what is there), you can create an arbitrary Calibration Table.

You will be prompted for `AMT/AREA` (or `AMT/HEIGHT`) in addition to the other table parameters, because, since you are not using peak data, the HP 3396 does not calculate the response factors for you. For all three calibration types, the integrator expects an absolute response factor. For an ISTD calibration, the instrument will calculate the relative response factor for each component at each level from the absolute numbers provided for the component and the ISTD peak at that calibration level.

Example `* EDIT CALIB 2 @`

```
1 = CALIB PROCEDURE
2 = RETENTION TIME WINDOWS
3 = TABLE ENTRIES
4 = PEAK GROUPS
5 = CALIB optionS

SECTION TO BE EDITED:
```

2. **Enter a section number and press [ENTER].**

The dialog for each section is the same as in the **[PREP] [CALIB]** dialog. When a section is done, the general menu above reappears. Select another section to edit or press **[ENTER]** to quit.

Response Factors for ISTD calculations cannot be edited.

Option 3: Calibration Options

The option 3 dialog lets you change some of the values in the calibration. This is the set described at the end of the **[PREP] [CALIB]** dialog. The changes are permanent, so that option 3 may be regarded as a quick editor for a specific set of entries. The dialog is shown here, but is explained in detail in *Preparing a Single Level Calibration* earlier in this chapter.

1. **Press [OP()] [3] [ENTER] to begin the option 3 dialog.**
2. **Respond to each of the dialog prompts by entering or retaining the desired values.**
3. **Press [BREAK] to abort the dialog.**

Example `* OP() 3 @`

```
CALIBRATION OPTIONS
RF of uncalibrated peaks [0.0000E+00 ]:
```
The numeric values in brackets are defaults.

```
Replace calibration fit [Y/N*]:
```
Y [ENTER]

```
P = point-to-point
L = linear (least square)
N = non-linear (quadratic)

Calibration fit [N/L/P*]:
Disable post-run RT update [Y/N*]:
ISTD peak #:
ISTD AMT [1.0000E+00 ]:
SAMPLE AMT [0.0000E+00 ]:
MUL FACTOR [1.0000E+00 ]:
```
These lines show up only if ISTD has been chosen in the **[PREP]** [**CALIB**]dialog.

The **Y** response to `Replace calibration fit` causes the printing of the lines up to `Calibration fit`. A response of **N** will skip those lines. The `ISTD peak #` value, above, is called `ISTD CAL#` in the **[PREP]** dialog and is requested in a different place in the dialog.

4. **Press [OP()] [−] [3] [ENTER] to reset the calibration to the default values.**

Listing a Calibration

Entire calibration files can be listed from either the active workspace or a disk.

1. **Press [LIST] [CALIB]** {*filespec*} **[ENTER] to list a calibration file.**

filespec specifies the device and file name (see Chapter 7). If *filespec* is omitted, the calibration currently in the active workspace is listed. If there is no calibration in the active workspace (and *filespec* is omitted), the HP 3396 prints `NO CALIB TBL.`

Example

```
* LIST: CALIB A:CALIB1 @   lists a calibration from disk drive A
          ISTD
          REF % RTW:   5.000   NON-REF % RTW:   5.000

LEVEL:  1     RECALIBRATIONS:  1
CAL#          RT         LV       AMT       AMT/AREA
  1&         0.082        1 8.0000E+01     2.1639E-04
                    & indicates reference and ISTD peak
  2                 R indicates reference peak
  3                 S indicates ISTD peak

CAL#          NAME
  1

CALIBRATION optionS
RF of uncalibrated peaks .... 0.000E+00
Calibration fit ............. P
Disable post-run RT update .. NO
ISTD peak # ................. 1
ISTD AMT .................... 1
SAMPLE AMT .................. 1.0000E+00
MUL FACTOR .................. 2.0000E+00
```

Deleting a Calibration

1. **Press [DEL] [CALIB]** {*filespec*} **[ENTER] to delete an entire calibration.**

This deletes the calibration described by *filespec* from a disk drive. If *filespec* is omitted, the calibration in the active workspace will be deleted. In both cases, the HP 3396 prompts

```
DELETE ALL [Y/N*]:
```

to ensure that you want to delete the file you have named.

Deleting Selected Levels

1. **Press [DEL] [CALIB]** *level number* **[ENTER] to delete the specified level from the calibration in the active workspace.**

Example `* DELETE CALIB 2 @`

Note: Level 1 cannot be selectively deleted.

Deleting Calibration Peaks

1. **Press [EDIT] [CALIB]** {*level number*} **[ENTER] to edit a calibration.**
2. **Type 3 to edit the calibration table.**
3. **Type a minus sign, the number of the peak you wish to remove from the table and press [ENTER].**

Example

```
*  EDIT  CALIB  2  @
1 = CALIB PROCEDURE
2 = RETENTION TIME WINDOWS
3 = TABLE ENTRIES
4 = PEAK GROUPS
5 = CALIB OPTIONS

SECTION TO BE EDITED: 3 [ENTER]
CAL#:  -1
```

Storing and Retrieving Calibration Files

The calibration resides in the active workspace of the HP 3396 until it is deleted or overwritten by a new one. A calibration can be stored individually or as part of a method. See Chapter 8 for more information about methods.

1. **Press [STORE] [CALIB]** *filespec* **[ENTER] to store a calibration.**

filespec specifies the device and file name for storage.

2. **Press [LOAD] [CALIB]** *filespec* **[ENTER] to recall a stored calibration to the active workspace.**

The HP 3396 adds a .CAL extension to the *filespec* if you don't, for both storing and loading.

About Sample Information

All the calculations, both calibrated and uncalibrated, include a Multiplication Factor. ISTD requires the identity and amount of the internal standard, and both ESTD and ISTD use a SAMPLE AMT value. These values can be set on a sample-by-sample basis using the sample information table (see chapter 9) or can be applied to all samples using option 3 or option 7. The SAMPLE AMTS are not the same as the amounts entered for the standards in the **[PREP] [CALIB]** dialog. If you enter a sample amount for a standard, you will obtain an ESTD% or ISTD% calculation instead of absolute values. Sample amount is the concentration or volume of the original sample in whatever units you wish to use.

The sample table is used when running a sequence or in manual analyses; the first question in the option 7 dialog sets this up. If data for a specific sample is found in the table, it will be used. If there is no data for the current sample, but data was found for a previous sample in the sequence, that data will be used (the assumption is that the current sample is a repeat of the previous one). If there is no match and no sample was previously matched, then the option 3 or option 7 value is used.

Option 3, option 7, and the calibration all store MUL FACTOR in the same memory location. When you change one of these entries, you change them all. Similarly, there is only one storage location for SAMPLE AMT whether you enter it in the calibration, option 3, or option 7—likewise for ISTD AMT. At the beginning of each analysis the value in the sample information table is loaded into this single memory location.

The only way to have different parameter values for different samples is through the sample information table.

Option 7: Default Sample Information Dialog

Option 7 is equivalent to a single entry in the Sample Information Table from the **[PREP] [SEQ]** dialog. The option 7 values are used when the Sample Information Table does not provide them. See chapter 9 for more details about the Sample Information Table.

Respond to each of the dialog prompts by entering or retaining the desired values. To abort the dialog, press **[BREAK]**. To retain an existing value, simply press **[ENTER]**.

1. **Press [OP()] [7] [ENTER] to begin the option 7 dialog.**
2. **Respond to each of the dialog prompts by entering or retaining the desired values.**
3. **Press [BREAK] to abort the dialog.**

Example `* OP() 7 @`

```
DEFAULT SAMPLE INFORMATION
USE SAMPLE TABLE IN MANUAL RUN [Y/N*]: Y [ENTER]
```

Using the sample table allows you to enter data for a set of samples in a sequence sample table, but use the information while doing manual runs.

Also answer **Y** if you want to use the Sample Table with an external sequencing device, i.e., something that supplies START signals through a Remote Control cable. Manual runs and externally sequenced runs appear the same to the HP 3396.

```
ISTD AMT [0.0000E+00 ]:
SAMPLE AMT [0.0000E+00 ]:
MUL FACTOR [1.0000E+00 ]:
RECALIBRATION [Y/N*]:
```

Respond **Y** to perform automatic recalibration (discussed later in this chapter) after the next run. If you respond **Y**, you will get a second prompt:

```
LEVEL [0   ]:
```

If you enter a positive number: Causes an averaged recalibration of the specified level. Response data from the next run will be combined with response data from all runs for this level since the calibration was created or replaced (see below); the calculation is a simple average. The calibration counter, called RECALIBRATIONS in the calibration listing, is increased by 1.

If you enter a negative number: Causes a replacement recalibration of the specified level. The new calibration is based only on the data from the next run. All previous data for this level are discarded and the calibration counter is set to 1.

```
NAME:
```

A 12-character sample name may be entered here. It will appear on the report.

```
REPORT MEMO:
```

A report notation of up to 126 characters may be entered.

4. **Press [OP()] [−] [7] [ENTER] to reset the sample information to the default values.**

Example

```
*  OP  #  -7
*  LIST:  OP  #  7

DEFAULT SAMPLE INFORMATION
USE SAMPLE TABLE IN MANUAL RUN ..... NO
ISTD AMT ............................. 0.0000E+00
SAMPLE AMT ........................... 0.0000E+00
MUL FACTOR ........................... 1.0000E+00
RECALIBRATION ........................ NO
```

SAMPLE NAME and REPORT MEMO are not included in the default information.

How Calibration Works

Chapter 5 contains a detailed explanation of calibrations.

When the detection sensitivity varies significantly between components or when sensitivity varies with the amount of a component, good quantitative results require the use of a calibrated calculation.

NORM is similar to AREA% or HEIGHT% except that the Measured Responses are corrected before calculating percentages. ESTD and ISTD can report in any units, not just percent. If a Sample Amount is supplied for these calculations, the results will be in percents of that amount.

A mixture contains 1 gram per liter of compound A and 1 g/l of compound B. The chromatogram shows 600 area units for A and 400 area units for B. Clearly the detector is more sensitive to component A than to component B. We can express these sensitivities as response factors:

$$\text{Response Factor} = \frac{\text{the known amount (or concentration)}}{\text{the Measured Response (area or height)}}$$

The factors for this example are 1.667E−3 (0.001667) g/l per count for A and 2.5E−3 (0.0025) g/l per count for B.

A sample containing both A and B yields peak areas of 840 and 730. This does not mean that there is more A than B. Multiply each Measured Response by its Response Factor (to correct for the difference in sensitivity of the detector to the two compounds), and we get 1.4 g/l for A and 1.825 g/l for B. There is more B than A.

Calibration measures the detector sensitivity for each peak and uses that information to correct the data obtained from sample runs. Uncalibrated peaks may be assigned a response factor or omitted from the calculations.

In many cases, a graph of Measured Response versus amount of component is a straight line passing through the origin. The correction of the data is simply

Corrected Response = Measured Response x Response Factor

as in the example above. When a component is nonlinear, the Response Factor changes with component amount. The conversion of Measured Response to Corrected Response is more complicated. A curve of some sort must be fit to the calibration data and used to correct the Measured Responses for detector behavior. This is done using a multilevel calibration, described in Chapter 5.

Unknown Peaks

It is common to find unidentified (uncalibrated) peaks in sample runs. A response factor for these peaks can be supplied as part of the option 3 dialog.

Option 4 controls the printing of uncalibrated peaks in reports. It is completely independent of option 3.

Retention Time Updating

The HP 3396 identifies detected peaks by comparing the actual retention times with the retention times in the calibration. Since times vary somewhat from run to run, it begins by locating designated reference peaks. If these “dependable” peaks have shifted, it assumes that similar shifts apply to all other peaks. It then calculates the expected (after allowing for shifts) retention times for the rest of the calibrated peaks and searches for them there.

Reference peaks are assumed to be prominent, “dependable” peaks. If several peaks are within a reference peak search window, the largest is identified as the reference peak. Overlapping windows are resolved by giving half the overlap to each window.

If all the reference peaks are detected, the HP 3396 updates the retention times in the calibration to reflect the changes in actual times. The updating is weighted in favor of the older retention times to minimize the effect of a single bad run. The relationship is

$$RT_{(n+1)} = [(0.75 \times RT_{a})] + [(0.25 \times RT_{n})]$$

where $RT_{(n+1)}$ is a new value to be stored in the calibration table for the next run.

RT_{a} is the current retention time value for a peak stored in the calibration table.

RT_{n} is the value observed in the last run.

Search windows for nonreference peaks are specified exactly as for reference peaks; however, there are important differences in how they are used:

- The search windows are centered on the calibration retention times after they have been adjusted for the shift revealed by the reference peaks.
- Because a component may be present in any amount, the peak closest to the adjusted retention time (center of the window) is identified when multiple peaks are within a window.

You can disable retention time updating through option 3 or an entry in the **[PREP] [CALIB]** dialog.

7

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Saving Data

Saving Data

Understanding Data Storage

The HP 3396 automatically stores operating parameters, data, and files created during normal operation in its internal memory. In addition, you can elect to save certain types of files, data and BASIC programs in internal and external data storage locations

Files are stored with “date stamps” that indicate the date and time they were created.

Where is Data Stored?

Data, files, and programs produced during integrator operation may be stored in four areas:

- in the HP 3396 internal memory (M:);
- on external HP-IL or HP-IB disk drives, connected to the HP 3396 via an HP-IL cable or the HP-IB/HP-IL Interface;
- on a disk volume of a data storage expansion card in slot 1 or slot 2 of the HP 3396 Series III;
- on a host computer system through RS-232-C data communications.

Internal Memory

The HP 3396's internal memory provides

- a temporary storage area for currently active operating parameters, such as run parameters, integration functions, options, etc. It also includes calculated results from the last run.
- a temporary storage area for peak data from the most recent run or reintegration.
- a semi-permanent storage area for signal data files, BASIC program files, and other files.
- a semi-permanent work area for developing and holding BASIC programs.

Power Failures

After a long power failure (greater than about 95 hours), the integrator performs a cold-start power-up when power is restored and assigns default values to all of its operating parameters. Information stored in the BASIC work area and Memory disk may be lost after at least 95 hours without power. For secure data storage, save your files on an external disk.

When a power failure is shorter than about 95 hours, the integrator performs a warm-start power-up when power is restored. Operating parameters, Memory disk, and the BASIC work area are preserved as they were before the power failure. This memory backup feature not only protects memory against power failures, it also allows you to move the unit without destroying the contents of memory.

The Active Workspace

The active workspace is the name given to the area of memory where the currently active parameters and set points in an HP 3396 system are stored. The active workspace always represents a complete set of operating parameters required for making an analysis. The active workspace consists of the following information:

- Method (including run parameter values, option dialog responses, and timetables)
- Processed peak data
- Calibration data
- Sequence (for run automation)
- System configuration information
- INET configuration table (lists active INET devices)
- INET instrument set points

When you first apply power to the integrator, the operating parameters in the active workspace are the default, or initial power-up values. As you set the unit up for various operations, you overwrite these default operating parameters with your own. A given set of operating parameters may be stored away and saved as a specific method file. Whenever you want to use that particular set of operating parameters again, the method file can be loaded (read) back into the active workspace. The operating parameters, calibration, or automation sequence for an analysis must reside in the active workspace before you begin the analysis.

If the integrator is configured with no peripheral devices, the active workspace is only a set of HP 3396 parameters. However, if the Hewlett-Packard Interface Loop (HP-IL) or Instrument Network (INET) is installed, the active workspace contains the configuration and set points of these instruments as well. See the HP 3396 *Reference Manual* for information about HP-IL and INET.

In addition, the active workspace contains processed peak data from the most recent run. The key sequence **[LIST] [LIST]** shows, among other things, the peak capacity. This number gives an indication of how much space is left in the active workspace for processed peak data.

The Memory Disk

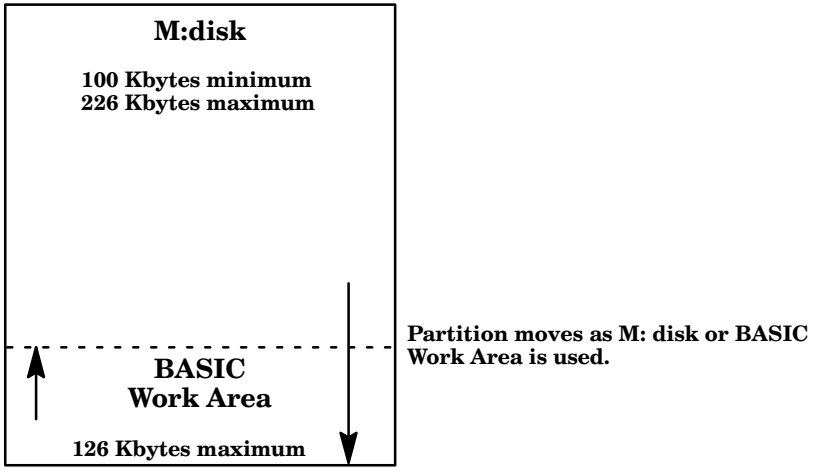
The Memory (M:) disk portion of the integrator's internal memory is reserved for storing BASIC programs, methods, sequences, calibrations, and run data as though they were being stored as files on an external medium. Files (other than data files) are not usable for analyses while they are residing on the M: disk or any external disk. They must be loaded into the active workspace in order to be used by the integrator.

The Memory (M:) disk shares its space with the BASIC program work area. (See figure below.) M: disk has a minimum size of 100 Kbytes. When the integrator is in default mode, 20 KBytes of space are reserved for BASIC programs. The reserved space can be made available for data storage with the Application Program *Maximizing Memory.* See *Using Application Programs* for more information. When no BASIC programs exist, the HP 3396 Series III M: disk can occupy 226 Kbytes of memory. (32 Kbytes are permanently reserved for system operations).

Since the memory area of M: and the BASIC work area share space, there may not be room to load a BASIC program when too many M: files exist. Similarly, there may not be room for data storage when a large BASIC program is present in the BASIC work area. In either of these cases, files may need to be copied to another disk, or purged, in order to load the new files.

After a long power failure (greater than 95 hours), or when a cold start occurs, information stored on the memory disk will be lost. Store data and files on external media for secure storage.

User-Accessible Memory



The E: Disk

The EPROM disk is a 128-Kbyte disk designed for storage of application programs. A standard set of these programs is included with the HP 3396 and discussed in *Using Application Programs*.

The BASIC Program Work Area

With BASIC installed, the BASIC program work area of internal memory allows you to create and run BASIC programs. This work area contains a maximum of 20 Kbytes of memory, which are shared with the Memory (M:) disk. As a result, the more BASIC program work area you use, the less that will be available for storing programs, operating parameters, and run data as files on the Memory disk. See figure above.

When exiting BASIC, you have the option of leaving your BASIC program in the BASIC program work area or deleting it. If you leave the program in the work area, the program will be readily accessible upon re-entering BASIC. However, deleting BASIC programs relinquishes the work area to the Memory disk. To clear the BASIC program work area and keep your program, save it as a BASIC file and then delete it from the work area. For more information about BASIC, refer to the HP 3396 BASIC Language Manuals.

Caution

Pressing the [CTRL] and [BREAK] keys simultaneously erases the contents of the BASIC work area.

The [CTRL] [BREAK] key sequence provides an intermediate restart (or cool-start) for the HP 3396. Although the contents of the BASIC work area are lost, all operating parameters are preserved, as are the contents of the Memory disk.

External Storage

Data, files, and programs from the integrator may also be stored externally on a host computer or on a “disk”. The disk can be a hard drive, the volume of an expansion card, or a hard or flexible (floppy) disk that is inserted into a disk drive. A disk is used to record or write data for data storage and to read the information back to the HP 3396. The disk drives that are compatible with the HP 3396 at the time of this printing are listed below. The HP 9153C Disk Drive contains a hard or fixed disk. The other drives listed use flexible or “floppy” disks.

Disk Drives Compatible with the HP 3396

Disk Drive Model Number	Notes
HP 9114A/B[1]	HP-IL, battery-operated, single floppy drive
HP 9122C/D	HP-IB device[2], dual floppy drive
HP 9153A/C[1,3]	10 Megabyte Winchester, HP-IB device[2]

Note: The HP 3396 is only compatible with double-sided *subset 80* command set disk drives.

[1] This disk drive is no longer available.

[2] Requires use of an HP 82169A HP-IL/HP-IB Interface.

[3] When used with the HP 3396, the HP 9153C 10-Mbyte disk drive should be divided into the maximum number of volumes. This will keep disk directory listings on the HP 3396 to a reasonable length. See the HP 9153C *Getting Started* Manual for instruction on setting the configuration switch to determine the number and size of volumes.

Data Storage Expansion Cards

The HP 3396 Series III has two slots for expansion cards in the back panel. It accepts cards that conform to PC Memory Card International Association (PCMCIA) PC Card Standard Release 2.1 dimensions. Supported cards can be Type I, Type II, or Type III.

WARNING Do not place expansion cards formatted by other computers in the HP 3396 Series III integrator. The data on the cards may be destroyed when the HP 3396 Series III attempts to identify the cards.

- Type I cards can be ATA FLASH, SRAM, or ROM cards.

  ATA FLASH cards employ solid state flash memory and emulate a hard disk. FLASH cards can be kept in the integrator and used as expansions to the internal RAM disk (M:). Since flash memory needs no batteries they can be removed and used to archive data. They provide complete protection from power failures, and are an ideal way to store data permanently.

  SRAM cards are used like a floppy disk or the internal memory of the HP 3396. They utilize a battery to maintain data storage in the event of a power failure to the HP 3396. The battery will remain good for many years as long as power is maintained to the integrator, and will provide protection against very long power failures. The status of the battery as “OKAY”, “REPLACE”, or “DEAD” is given in every directory listing that involves an SRAM card, so sufficient warning is given to prevent data loss before the battery fails. However, if an SRAM card is removed from the integrator, the battery must be changed periodically. Since the data is lost if the battery is removed without maintaining power to the card, the card must be inserted into the integrator, or another power source, when the battery is changed. For this reason, SRAM cards are best kept in the HP 3396 and used as expansions to the internal RAM disk (M:) of the integrator, rather than removed for permanent data storage.

  ROM cards maintain data integrity without a battery. They cannot be divided into volumes or used for data storage. ROM cards may contain programs used with the HP 3396.

- Type II cards are ATA FLASH cards with memory capacities of 40 Mbytes or greater. Other than size they are indistinguishable from the smaller capacity Type I ATA FLASH cards.
- Type III cards are 1.8 inch hard disk drives. Hard disk drives do not utilize batteries, so they can be used to store data permanently. Because Type III cards can store so many files, directory operations can be cumbersome. However, they are an economical way to store large amounts of data.

Host Computer

A host computer can be connected to the HP 3396 with an RS-232 cable. The host can be used to write BASIC programs directly on the integrator from the computer keyboard in external BASIC, and the host computer facilities can be used for file storage and manipulations. See the *Reference Manual* for more information on using a host computer.

The Hewlett-Packard Peak-96 Information Manager is a software package that enables all HP 3396 operations to be controlled by a host computer, and provides an easy interface for storing data and manipulating files. The Hewlett-Packard ChemServer is another software package that allows HP 3396 data to be stored and manipulated on a personal computer. For more information about Peak-96 and the ChemServer, contact your HP representative.

Preparing External Media for File Storage

The HP 3396 memory disk, designated M:, is ready to store data immediately. The HP 3396 Series III has 226 Kbytes of RAM (Random Access Memory) capacity that is available to the user.

Before data can be stored on an external medium (disk drive or data storage expansion card), the medium must be prepared to receive and store the data. Expansion cards must be divided into disk volumes. Hard disk drives are usually divided into volumes manually. Each disk volume of an expansion card or hard disk drive, as well as floppy disks, must be formatted before use.

Preparing Data Storage Expansion Cards for Use

WARNING Always turn the HP 3396 Series III OFF with the ON/OFF switch before inserting or removing expansion cards.

Data storage expansion cards must be divided into volumes before use. There can be 1, 2, 4, 8, or 16 volumes per card. Each volume is assigned an individual disk specifier (A:, B:, etc.) by the integrator. The total card area is divided evenly between the number of volumes selected. A larger number of volumes results in faster file retrieval times and shorter pack time; most analysts divide the disk into 2 to 8 volumes, depending on the card size, and use each one for a different project. Each volume is formatted like a floppy disk in a disk drive.

The user divides the card into volumes with the SSET VOL command. The SSET VOL command is illustrated below.

Type **SSET VOL SLOTx,n** **[ENTER]**

x=the slot on the integrator containing the card being divided into volumes—slot 1 or slot 2

n=the number of volumes desired—1, 2, 4, 8, or 16 volumes

The HP 3396 Series III automatically assigns disk specifiers to the volumes on the data storage expansion card. The integrator assigns letters alphabetically, starting with the disks in configured disk drives or hard disks, then proceeding to the volumes on the card in slot 1 and then in slot 2. The volume specifiers can be A through Z, excluding H, M, E, O, and Q. There are 21 total volume specifiers available. If you divide your media into more total volumes than 21 (for instance, two expansion cards of 16 volumes each is too many) the integrator will divide the media into the requested number of volumes, but since some of the volumes won't have a specifier, they will be unavailable for use. Be sure to have no more than 21 total volumes, including floppy disks.

The SYSTEM command will show the card type and slot of any memory expansion cards present, and the disk specifiers that were assigned after a SSET VOL command. A typical output in response to the SYSTEM command is shown on the following page.

Example Type **SY [ENTER]**

```
ADDRESS   DEVICE ID                   ACCESSORY ID & CLASS
8         HP82169A                    43H INTERFACE
1-7       RESERVED FOR HPIB DEVICES
SLOT1                                 2MB-SRAM
SLOT2                                 512KB-FLASH

DISC NAME     ADDRESS     DRIVE#      VOLUME ID     MEDIA
A             7                       EPA1
B             7                       WATER
C             SLOT1                   CLWAT         FLASH
D             SLOT1                   METHOD        FLASH
F             SLOT1                   AUG           FLASH
G             SLOT1                   SAVE          FLASH
I             SLOT2                   TEMP          SRAM
J             SLOT2                   TEMP2         SRAM

RS-232-C SWITCH SETTINGS

Baud                    4800

Timeout                 15 sec

Handshake Delay         Disabled

Hardware Handshake      Enabled
```

In this example, the user has partitioned the data storage expansion card in slot 1, which is a 2 MB FLASH card, into four volumes; each volume will have a capacity of 512 KB (2MB/4). The slot 2 SRAM card was divided into two volumes. The disk specifiers of the volumes in slot 1 have been assigned as disks C, D, F, and G. The disk specifiers I and J are the disk specifiers of the card in slot 2. A dual disk drive is present; the floppy disks are A and B. The volume ID is the name the user gives the disk during the format operation.

Note that the disk specifiers the HP 3396 assigns to the volumes of memory cards depend on which slot holds the card. The integrator starts at the beginning of the alphabet and assigns the first unused letters to the volumes on the card in slot 1, then continues on to the card in slot 2, if there is one. Therefore, if the cards are moved to different slots, the disk volume specifier assignments on each card will be changed. The data will still be intact, but will seem to be located on a different disk.

In default mode, the integrator assigns the first disk specifiers to the floppy disks when both a disk drive and expansion cards are present. The alphabet continues with the expansion card disks. The SSET CARD_FIRST command changes the default mode so that disk specifiers are first assigned to the expansion card volumes.

To change the default status, type:

SSET CARD_FIRST [ENTER]

Now a SYSTEM command will show the card volumes assigned to the first letters of the alphabet.

Example: **SY [ENTER]**

```
ADDRESS   DEVICE ID                  ACCESSORY ID & CLASS
8         HP82169A                   43H INTERFACE
1-7       RESERVED FOR HPIB DEVICES
SLOT1                                2MB-SRAM
SLOT2                                512KB-FLASH

DISC NAME     ADDRESS     DRIVE#      VOLUME ID     MEDIA
A             SLOT1                   CLWAT         FLASH
B             SLOT1                   METHOD        FLASH
C             SLOT1                   AUG           FLASH
D             SLOT1                   SAVE          FLASH
F             SLOT2                   TEMP          SRAM
G             SLOT2                   TEMP2
I             7                       EPA1
J             7                       WATER

RS-232-C SWITCH SETTINGS

Baud                    4800

Timeout                 15 sec

Handshake Delay         Disabled

Hardware Handshake      Enabled
```

To return to the default mode, with floppy disks assigned the first volume letters, type:

SSET HPIL_FIRST [ENTER].

The FORMAT Command

For all external media (disks in disk drives, volumes of data storage expansion cards, and volumes of hard drives), the next step in preparing for file storage is to initialize or format the disk. The FORMAT command initializes a disk by checking it for damage and partitioning it into labeled sections for accepting data.

Caution The FORMAT command erases any data currently on the disk. Copy all important files to another disk before using this command.

1. Insert a floppy disk into the appropriate disk drive if you are formatting a floppy disk. If you are using an expansion card or hard disk, be sure to divide it into volumes first.
2. Type **FORMAT A:, DISKA, 200 [ENTER]**

   This will format the disk specifier A, name it DISKA, and allocate directory space for 200 files. The name must be six characters or less. The number of files depends on the size of the disk; 100 to 200 is usually used for a floppy disk. When formatting hard disks or volumes of a data storage expansion card, 200 files per megabyte are usually adequate.

Sector Sparing

When you format the volumes of a magnetic expansion card (that is, a hard disk expansion card), you can invoke an optional sector sparing process. Sector sparing is a magnetic disk defect management process that will identify and relocate bad sectors. Sparing should not be necessary the first time you format a hard disk because most manufacturers spare disks before selling them. After using a magnetic disk, disk errors may occur which render the disk unusable. Sector sparing relocates the bad sectors so the rest of the disk can be used. The HP 3396 takes about one minute per megabyte to spare a disk.

Only hard disks should be spared. The integrator ignores the “S” if a SRAM or ROM disk is in the slot; a flash disk will be spared if the user requests it, but it never needs to be, and sparing just wastes write cycles. To invoke sector sparing, type:

FORMAT A:, DISKA, 200, S [ENTER]

After closing out the command with the [ENTER] key, the HP 3396 prints out a start and stop time stamp as follows:

SECTOR SPARING START—mmm,dd,yyyy, hh:mm:ss

WAIT IS ABOUT 10 MIN/1MB

SECTOR SPARING END—mmm,dd,yyyy, hh:mm:ss

After finishing the sector sparing operation, the HP 3396 proceeds to format the disk.

Since sector sparing is an optional part of the format process, the prompt that the HP 3396 prints in response to a formatting error includes the “S”. The prompt is shown below.

```
FORMAT WANTS: DISK, NAME, MAX FILES, (S)
```

Just ignore the “S” unless you are formatting a magnetic disk for the second or subsequent times.

The FORMAT command can be used to erase all files on a disk.

Format can also be executed through BASIC. See the HP 3396 BASIC Language Manuals for details.

Once you have formatted the volumes on an expansion card the HP 3396 Series III should recognize the volumes after a power fail, coldstart, or control break. If you are ever in doubt as to whether the correct card volumes are recognized, use the SYSTEM command to force the integrator to re-identify the disks.

Specifying Files for Storage and Retrieval

The integrator stores information in files. A file is a collection of data organized for easy storage and retrieval.

Files are named by the user or the instrument and stored internally on the memory disk, on an external disk, or on a host computer system. A file specifier, or *filespec*, completely designates a particular file.

Example **A:METH625.MET**

A *filespec* has the form *d*:*filename.ext*

Identifying the Disk

d: is the disk specifier (abbreviated *diskspec*) and consists of a disk drive name followed by a colon (:). If it is not specified, the default *diskspec* is used. At power-on the default *diskspec* is M:. Legal disk drive names are the capital letters A through Z, with these constraints:

- H: names the host computer connected to the “RS232” receptacle on the rear panel,
- M: names the HP 3396 Memory disk, and
- E, O, and Q may not be used.

The default *diskspec* may be changed by typing in a disk drive name followed by a colon.

1. **Type A: [ENTER] at a system prompt (*) to make disk drive A the default drive.**

Example `*A:`

Now when you want to store files to the A: disk drive, no diskspec is necessary. To specify the internal memory, **M:** now must be specified.

2. **Type [S] [Y] [ENTER] to determine which external disks are configured for file storage.**

Identifying The File

filename is the name of a file. It may contain up to 8 characters if a file extension is used (see below) or 10 characters without an extension. Some commands and keywords add a default extension automatically if none is provided.

Identifying the File Type

.*ext* is a file extension consisting of a period (.) followed by a three-letter file type code. Note that file extensions do not guarantee the contents of a file. The **COPY** and **RENAME** commands may be used to change or eliminate the file extension on a file without affecting the contents. For example, changing the file M:SIGNAL.BNC to M:MYFILE does not invalidate its use as an input file for reintegration. The extension is only guaranteed valid at the time a file is automatically created by the integrator. It is rarely necessary, however, to rename or remove file extensions.

Valid extensions and file descriptions are shown below. Files with .BAA, .RPT, .RPA, .UA1, .UA2, and .UA3 extensions are LIF ASCII files (Logical Interchange Format, ASCII encoded character data). All others are LIF binary files with contents unique to the HP 3396.

HP 3396 Valid File Types

File Extension	File Type
.BAA	BASIC program file
.BAS	BASIC program file in memory image format
.BNA	Bunched data file from an **ANALYZE** command
.BNC	Bunched data file from an analytical run
.CAL	Calibration file
.DAT	BASIC data file
.MET	Method file
.PRA	Processed peak file from an **ANALYZE** command
.PRO	Processed peak file from an analytical run
.SEQ	Sequence file
.RAW	Unbunched signal data file
.RPA	Report file from an **ANALYZE** command
.RPT	Report file from an analytical run
.UA1	User-defined ASCII file
.UA2	User-defined ASCII file
.UA3	User-defined ASCII file
.UD1	User-defined file
.UD2	User-defined file
.UD3	User-defined file

Saving Data from a Run

Data from a run *must* be saved if you want to reanalyze the data later. You may specify storage of signal data through the **[PREP]** or **[EDIT] [METH]** dialogs or through option 2.

Example

```
*  OP # 2
RUN DATA STORAGE
Store signal data [Y/N*]: [Y] [ENTER]
Device [M*]:[H] [ENTER]
Bunched or raw data [B/R*]:[B][ENTER]
Local run-time storage [Y/N*]:[ENTER]
Keep run-time storage [Y/N*]:[ENTER]
Store processed peaks [Y/N*]:[ENTER]
```

1. **Press [OP()] [2] [ENTER] to begin the option 2 dialog.**

```
*  OP # 2

RUN DATA STORAGE
Store signal data [Y/N*]:
```

2. **Type Y to store signal data.**

```
Device [M*]:
```

3. **Press [ENTER] to retain current storage device or enter appropriate letter for a different storage device.**

If you specify signal data storage to the Memory (M:) disk, the integrator uses the filename SIGNAL to store the data. The proper extension (.RAW, .BNC, or .BNA) is appended. M: is the device specifier. A typical complete file specifier for signal data storage on the Memory disk is `M:SIGNAL.BNC` for bunched data from a run.

Caution

Only one file named SIGNAL with a .RAW, .BNC or .BNA extension may exist on the Memory disk at any time. When you begin a new run, SIGNAL.RAW, SIGNAL.BNC, and SIGNAL.BNA files are purged. The integrator warns you of this condition by printing the message

CAUTION: Previous signal data will be lost

in the option 2 dialog. Before beginning a run, RENAME the previous run's SIGNAL file to a new filename if you want to save the data for future use. See *Renaming Files Automatically* **in** *Using Application Programs***.**

Bunched signal data stored during a reintegration will *not* overwrite data from a prior run because the integrator uses the extension .BNA instead of .BNC for signal data generated through reintegration, effectively changing the file's name. Subsequent reintegrations will overwrite the .BNA files, however, as the CAUTION above warns.

Raw vs Bunched Data

```
Bunched or raw data [B/R*]:
```

4. **Select bunched or raw data storage.**

Raw data will be stored by default when you choose to store signal data. On M: disk there is enough storage space for about 75 minutes of raw data. Bunched data takes less storage space and less time to reintegrate than raw data. At peak width 0.04, about 5 hours of bunched data can be stored on the HP 3396 Series III M: disk.

During reintegration, only signal data bunched by the PK WD function as part of the reintegration process (file extension .BNA) can be saved. The signal being input to the reintegration process cannot be stored because it is already coming from a previously stored data file.

If you specified a storage device other than a host computer, the dialog skips to "`Store processed peaks`"; see step 7 on the next page. If you specified device **H:** above, you will be asked

```
Local run-time storage [Y/N*]:
```

5. **Select storage to a host computer during or after the run.**

Pressing [**ENTER**] keeps no run-time storage; signal data is sent to the host during the run.

Pressing **[Y]** stores signal data in a file and sends it to the host computer after the run. The message `Copying signal file to host` appears after the run. You will be prompted for a device (other than H) where the file will reside before being sent to the host. (See CAUTION above about overwriting of previous reintegration files.) After the device prompt, you will be asked

```
Keep run-time storage [Y/N*]:
```

6. **Choose to keep or delete local signal file after transmission.**

Pressing **Y** keeps the local copy of the data as well as sending it to the host computer.

Pressing **N** deletes the local signal file after the run. The message `Deleting local signal file` appears after the run.

Caution **Two files with the same filename may be created when you reintegrate .BNA files and specify local run-time storage without keeping a local copy. Change the name of the input signal file using the COPY or RENAME commands to avoid deleting it when the local signal file of the same name is deleted. See** *Renaming Files Automatically* **in** *Using Application Programs***.**

Storing Processed Peaks

```
Store processed peaks [Y/N*]:
```

7. **Choose processed peak storage.**

Processed peaks are the retention time, area, peak type, and baseline information about each peak.

By default, the instrument will not store processed peaks. If you have a use for this information and choose to store processed peaks, you will be prompted for a storage device. Processed peak information is used by the Baseline program and by the HP Peak-96 Information Manager. Processed peaks may also be retrieved using a BASIC program.

See the *Plotting a Baseline Program* section of *Using Applications Programs* for related information.

Saving Other Types of Files

Calibrations, Methods, and Sequences

Calibrations, Methods, and Sequences are saved using the **[STORE]** key and the appropriate [METH], [SEQ], or [CALIB] filespec.

Example `* [STORE] [METH] C:METH20.MET`

In this example, the current method in the active workspace is named METH20 and stored on the C: disk drive.

See chapters 5, 8, and 9 for related information.

Reports

Reports are saved using the option 5 dialog.

Example
```
*  OP  #  5
PRINT & POST-RUN LIST OPTIONS
Large font [Y*/N]:[ENTER]
Store post-run report [Y/N*]:Y [ENTER]
Device [M*]:H [ENTER]
```

See chapter 10 for related information.

BASIC Files

While in BASIC mode, BASIC programs and data may be stored for future use with the **SAVE** command.

See the HP 3396 BASIC Language manuals for related information.

Listing the File Directory

A directory is the table of contents for the files stored on a disk. Use the **DIRECTORY** system command to determine

- the current names, types, and lengths of files
- the amount of space taken up by the files present
- the amount of space left on the HP 3396 Memory (M:) disk, or an external disk.

1. **Type [D][I]** *diskspec* **or** *filespec* **and press [ENTER] to get a directory listing.**

Example

```
*DI
          VOLUME NAME: MDISK        DRIVE: M
          DATE: JAN  8, 1995   15:20:13

  FILE NAME       LENGTH      CREATED/VERSION
SIG_BUFF.RAW        2048      01/05/90 14:48:02
LU1       .MET       256      01/03/90 18:04:45
PROG      .BAS       256      01/05/90 15:07:35

          USED          FREE          MAX
FILES        3            27           30
BYTES     2560          96768       100096
```

When a *diskspec* is indicated, the integrator lists all the files on the specified drive along with information about the number of files and bytes of free space available. The integrator responds with information about a particular file when a *filespec* is entered.

Terms used in the DIRECTORY listing are described below.

LENGTH—is the file's maximum length in bytes.

FILES USED—is the number of files on the disk. `FREE` is the number of entries left in the directory. `MAX` is the maximum number of files in the directory. After a **PACK** operation and after initial formatting, `FREE + USED = MAX`.

BYTES USED—is the total of numbers in the `LENGTH` column. `FREE` is the free space at the end of the disk. `MAX` is the maximum number of bytes on the disk, including the directory. After a **PACK** operation and after initial formatting, `FREE + USED < MAX`.

For external disks, the sum of the `USED` and the `FREE` columns for either files or bytes often does not add up to the `MAX` column. This is normal because the integrator does not reuse disk space automatically when a file is purged. See the subsection *Consolidating Storage Space* later in this chapter for instructions about repacking the files on a disk to recover the lost space. The M: disk is always packed automatically.

The example above illustrates how the integrator sets aside a special file on the Memory disk called M:SIG_BUFF.RAW. It is used as a temporary staging area (i.e. a buffer) for signal data being sent to external storage devices, such as disk drives. This file is always recreated after a run to make it available for the next run.

If the diskspec indicated is a volume of an SRAM card, the status of the SRAM battery is indicated as part of the directory heading as follows:

Example ***[D][I] [ENTER]**

```
VOLUME NAME:  ADisk DRIVE:  A

DATE:  June 10, 1994

SRAM BATTERY SLOT 1   (OK, REPLACE, or DEAD)
```

The directory listing continues as it would for any disk.

If the battery is dead, the disk data may not be intact; REPLACE means the battery is low, but the disk data is okay. You must maintain power to the SRAM card while changing the battery, or the data will be lost. You can leave the card in the HP 3396, or place it in another power source. The power source need not be logically compatible with the HP 3396 expansion cards; you could use a personal computer with expansion card slots.

2. Press [S][Y][ENTER] to list the system configuration, the address and disk specifiers for each external disk drive, and the slots, card type, and disk specifier of any expansion cards.

*** [S] [Y]**

```
         SYSTEM CONFIGURATION

ADDRESS   DEVICE ID   ACCESSORY ID & CLASS

-------   ---------   --------------------
      8   HP6890A       71H  INET
      9   HP82169A      43H  Interface
    1-7   (Reserved for HPIB devices)

 SLOT1    1MB-SRAM

 SLOT2    1MB-SRAM

DISC NAME  ADDRESS  DRIVE #  VOLUME ID  MEDIA

---------  -------  -------  ---------  -----
    A      7    0      0
    B      7    1      0
    C      SLOT1         0 TEMP    SRAM
    D      SLOT1         1 DDISK   SRAM
    F      SLOT1         2 FDISK   SRAM
    G      SLOT1         3 GDISK   SRAM
    I      SLOT1         4 IDISK   SRAM
    J      SLOT1         5 JDISK   SRAM
    K      SLOT1         6 KDISK   SRAM
    L      SLOT1         7 LDISK   SRAM
    X      SLOT2         0 XDISK   SRAM
    Y       SLOT2        1 YDISK   SRAM
RS-232-C SWITCH SETTINGS

Baud           9600

Timeout        15 sec

Handshake Delay              Off

Hardware Handshake  Disabled

*
```

Copying and Renaming Files

The **COPY** system command duplicates a file. This command allows you to rename and store a file to another disk.

1. **Type COPY** *filespec*, *new filespec* **and press [ENTER] to copy a file.**

Example `*COPY M:CA1.CAL,A:CA1.CAL`

Makes a copy of the calibration file CA1 on the A: disk.

The nature of the contents is unchanged in the copy, even if a different extension or no extension is specified. The creation time and date of the original file are preserved in the copy.

2. **Type RENAME** *filespec*, *new filespec* **and press [ENTER] to change the name of a file on the same disk.**

Example `*RENAME M:SIGNAL.BNC,M:RUN5.BNC`

Identifies the bunched signal file on the M: disk as data from Run 5.

The contents of the file are unaffected by a change in name or extension. The filename and extension of the original file must be specified in full.

See *Renaming Files Automatically* in *Using Application Programs* for related information.

Consolidating Storage Space

The first step in consolidating storage space is to delete any unnecessary files from memory.

1. **Type PURGE** *filespec* **and press [ENTER] to purge a file from memory.**

The complete name and extension must be entered.

Example `*PURGE A:METH1.MET`
METH1.MET is deleted from the A: disk.

Caution **Be sure that the file named is the one you really want to delete.**

After unnecessary files have been purged, use the **PACK** system command to consolidate storage space on an external magnetic disk drive and recover directory space. This command recovers disk space lost through normal data storage and file generation and deletion operations.

2. **Type PACK** *diskspec* **and press [ENTER] to pack a disk.**

Example `*PACK A:`
The Memory disk (M:) is packed automatically as necessary.

Caution

Do not interrupt the PACK operation. Corruption of data on the specified drive may result. Do not PACK disks if interruptions are likely (power failures or user actions), without making copies of important files first. When packing a disk on an HP 9114A/B Disk Drive, make sure the battery is adequately charged before beginning the PACK operation.

The PACK operation will not commence if the M: disk is full. If M: has no space, delete some files from M: before attempting to pack a disk.

8

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Using Methods

Using Methods

Before You Prepare a Method...

Before creating a method for a particular analysis, decide on the appropriate operations and settings for the run.

1. **Choose the calculation you wish to use.**

Some calculations (Normalization, ISTD, ISTD%, ESTD, or ESTD%) require that calibration be done. If you choose an AREA% or HEIGHT% report (via the **[PREP]** or **[EDIT] [METH]** or option 4 dialog), go to step 4.

2. **Run the sample to get the calibration information necessary for the desired report.**

3. **After analyzing the calibration sample, create the calibration.**

All parameters entered via the **[PREP]** or **[EDIT] [CALIB]** dialog automatically become part of the currently active method.

4. **Choose run and chart parameters.**

- zero
- attenuation
- chart speed
- area reject
- threshold
- peak width

These parameters may be entered for the entire run or programmed to change during a run by creating a timetable event. See chapter 2 for more information about time programming.

5. **Choose the necessary integration functions and external events.**

Instructions on how to time program integration functions can be found in chapter 1. Integration functions are listed in chapter 3. The timetable for external events is discussed in chapter 9.

Learning the Mechanics of Method Dialogs

As you work through a method dialog, you will be asked to enter information and to make choices. When a selection of choices is given, one of the possible responses will be followed by an asterisk. The "*" marks the current selection. If the current selection is the one you want, press **[ENTER]**. If you want to change the current selection, enter the letter corresponding to the desired entry.

Example `HEIGHT% report [Y/N*]:`

N (No) or AREA% is the current selection (indicated by the asterisk). Press [**ENTER**] to keep it.

Y (Yes) selects HEIGHT%.

Example `ENTER PLOT TYPE [S/F*/U/N]:`

F (Filtered plot) is the current selection. Press [**ENTER**] to keep it or press one of the alternative selections and press [**ENTER**].

S (Source plot) is an alternative selection.

U (Unigram) is an alternative selection.

N (No plot) is an alternative selection.

A method dialog can be ended at any time with the **[BREAK]** key. All set points entered before the **[BREAK]** key are retained. All entries not reached before the **[BREAK]** key was pressed keep the values they had before the dialog began.

Preparing a Method

The **[PREP] [METH]** dialog provides a formal all-inclusive procedure for establishing active workspace values. Specific parameters may be set directly using the **[EDIT] [METH]** or option dialogs.

Once a method is established in the active workspace, it must be stored for future recall. Subsequent use of **[PREP] [METH]** or an option dialog will overwrite the method in the current workspace.

1. **Press [PREP] [METH] to begin the method preparation dialog.**

Run Parameters

```
*  PREP  METH

RUN PARAMETERS
ZERO      [ 0    ]:
ATT 2^    [ 0    ]:
CHT SP    [ 1.0  ]:
AR REJ    [ 0          ]:
THRSH     [ 0    ]:
PK WD     [ 0.04 ]:
```

2. **Enter a new value for each parameter following the colon prompt or press the [ENTER] key if you wish to keep the current value.**

For each run parameter, the current value is shown in brackets. Run parameters are described in chapters 2 and 3 of this manual.

Timetable Events

```
TIMETABLE EVENTS
DELETE CURRENT TABLE [Y/N*]:
```

3. **Type Y to delete the current timetable. Press [ENTER] to retain the current timetable.**

If **Y** is entered above or a table does not exist in the current method, the integrator will prompt with

```
TIMETABLE EVENTS
SELECT EVENTS FROM THE FOLLOWING MENU
[IF/EX/ZE/^Z/AT/CS/AR/TH/PW/ST]

TIME:
```

4. **Type the run time at which you want a particular event to occur and press [ENTER] or press [ENTER] to skip timetable events and continue with step 8.**

If any timed events exist in the current method, the integrator will print

```
EVENT:
```

5. **Type the abbreviation for the event you wish to program and press [ENTER].**

IF is integrator function
EX is external event
ZE is plot zero
^Z is **[CTRL] [ZERO]**

The ^Z event is equivalent to pressing **[CTRL][ZERO]** during Unigram Mode. See chapter 4 for more details on its use. For entry as a Timetable Event through method preparation, however, ^Z is entered as the character pair ^ (caret) Z. The **[CTRL]** key is *not* used in the method dialog.

AT is attenuation
CS is chart speed
AR is area reject
TH is threshold
PW is peak width
ST is stop

```
VALUE:
```

6. **Enter a new value for the timetable event (if appropriate) and press [ENTER].**

^Z ([**^] [ZERO]**) and ST ([**STOP**]) do not require values.

```
TIME:
```

7. **Enter the time, the event to be programmed, and the value for each event you wish to time program. Press [ENTER] without a parameter to end the timetable.**

Example

```
TIME:   .30 [ENTER]
EVENT:  PW [ENTER]
VALUE:  05 [ENTER]

TIME:   8 [ENTER]
EVENT:  ST [ENTER]
VALUE:  [ENTER]

TIME:   240 [ENTER]
EVENT:  EX [ENTER]
VALUE:  3 [ENTER]
```

For each event to be edited, you are asked to specify the time, the event, and the new value (as shown). Inappropriate entries will result in the ? prompt. If a value is entered for a nonexistent function or parameter, an error message will result.

To delete an entry, precede the time of the event to be deleted with a minus sign **[–]**. To exit the dialog, press **[ENTER]** following the time prompt.

Time programming is described in chapter 2. The External Events Timetable is discussed in chapter 9.

Calibration File

```
REPLACE CURRENT CALIBRATION [Y/N*]:
```

8. **Press [ENTER] or N to retain the current calibration; press Y to change the calibration.**

This prompt is printed only if a calibration is already part of the current method, otherwise you will be asked

```
CALIBRATION FILENAME:
```

9. **Enter the name of the calibration file you wish to use for this method or press [ENTER] if a calibration is not part of this method.**

A calibration must already exist in the active workspace or in a stored file to be included in a method. The integrator will first establish if calibration is included in the current method.

Example `CALIBRATION FILENAME:` **A:CALB1**

The calibration parameters in A:CALB1.CAL now become part of the method file. If the filename entered does not exist or no filename is entered in response to the `CALIBRATION FILENAME:` prompt, *no* calibration parameters will be included in the current method. An AREA% report is printed unless an appropriate calibration exists in the active workspace.

You can prepare a calibration after preparing a method by way of the **[PREP] [CALIB]** dialog. The calibration then resides in the active workspace and will automatically be used by the method when no calibration is specified. This calibration is included if you later choose to store the method.

See chapter 5 and 6 of this manual for additional information concerning calibration.

Integration Plot Type

```
INTEGRATION PLOT TYPE
(Source/Filtered/Unigram/No Plot)
ENTER PLOT TYPE [S/F*/U/N]:
```

10. **Enter the appropriate letter for the desired integration plot type or press [ENTER] to retain the current selection.**

Integration plot type affects plot presentation only and may also be accessed through option 1.

S is for Source plot
F is for Filtered plot
U is for Unigram
N is for No plot selection

The No plot selection will suppress plotting and is useful during reintegration. Unigrams and integration are discussed in detail in chapters 3 and 4, respectively, of this manual. See chapter 2 for a description of plot types.

```
Presentation plot [Y/N*]:
```

11. **Press Y [ENTER] for improved plot quality.**

See chapter 2 for more information about selecting the plot quality.

Run Data Storage

```
RUN DATA STORAGE
Store signal data [Y/N*]:
```

12. **Press Y to store signal data or press [ENTER] or N for no data storage.**

Storing data allows you to reprocess it without repeating the analysis.

Example

```
Device [M*]:                                  [ENTER]
CAUTION: Previous signal data will be lost
Bunched or raw data [B/R*]:                   [B] [ENTER]
Store processed peaks [Y/N*]:                 [ENTER]
```

13. **Type the appropriate letter to indicate what data will be stored and where or press [ENTER] to retain the current selection.**

The responses shown to the dialog above store bunched run data to the internal M: disk; processed peaks are not stored. This dialog may be accessed directly through option 2. See chapter 7 for a more detailed discussion of data storage requirements.

Report Options

```
REPORT OPTIONS
```

14. **Press Y or N to choose the appropriate report options, or press [ENTER] to retain the current selection.**

Example

```
Suppress local report [Y/N*]:                 [ENTER]
HEIGHT% report [Y/N*]:                        [ENTER]
Replace report title [Y/N*]:                  [Y] [ENTER]
Report title:                                 PERFUME ANALYSIS
Replace amount label [Y/N*]:                  [ENTER]
Report uncalibrated peaks [Y/N*]              [ENTER]
Extended report [Y/N*]:                       [ENTER]
```

This section of the dialog permits you to suppress the report produced on the integrator after a run, print a HEIGHT% report, report uncalibrated peaks, or produce an extended report. This dialog may be accessed directly using option 4. The responses shown to the dialog above keep all the default values for these parameters except for the report title.

See chapter 10 for a more detailed discussion of report options.

Postrun List Options

```
PRINT & POST-RUN LIST OPTIONS
```

15. **Press Y or N to choose the appropriate postrun list options or press [ENTER] to retain the current selection.**

Example

`Large font [Y*/N]:`	**[ENTER]**
`Store post-run report [Y/N*]:`	**[Y] [ENTER]**
`Device [M*]:`	**[A] [ENTER]**
`External post-run report [Y/N*]:`	**[ENTER]**
`List run parameters [Y/N*]:`	**[Y] [ENTER]**
`List time table [Y/N*]:`	**[Y] [ENTER]**
`List calibration table [Y/N*]:`	**[ENTER]**
`List remote method [Y/N*]:`	**[ENTER]**
`Form feed before report [Y/N*]:`	**[Y] [ENTER]**
`Form feed after report [Y/N*]:`	**[ENTER]**
`Skip perforations in report [Y/N*]:`	**[ENTER]**

This section of the dialog permits you to specify if you want to store a report and where. You may also elect to list the run parameters, timed events, the calibration table, and the remote method after the report. In addition, you can choose to form feed before and after a report and skip perforations during reports and plots. The same dialog may be accessed via option 5. The responses shown to the dialog above store the report to the A: disk, list run parameters and timed events after the report, and do a form feed before the report.

See chapter 10 for a more detailed discussion of postrun list options.

Range Setpoints

16. **Press [ENTER] to retain the current setpoints.**

```
C1 Range [5]              [ENTER]

Buffer Range [5]          [ENTER]
```

The Range Setpoints are used only with an HP 6890 GC and HP 3396 Series III integrator. The HP 6890 is capable of producing data that is 37 bits wide, but the HP 3396 integrator can accept only 32 bits. The range parameter allows the user to select the data bits the HP 3396 will use in generating a report. Range 0 starts with the lowest data bit and takes the lowest 32 bits available; range 1 starts at data bit 2 and goes to bit 33, and so on. Range 5 takes the data from bits 5 through 37. Range 5 includes the data range that is generated by the HP 5890, and is the default value.

Selecting Range 5 will result in peak areas similar to those produced by the HP 5890. Selecting a lower range will result in larger peak areas, since the lower data bits are more sensitive. Changing to lower ranges may be helpful when working with extremely small or dilute samples.

The buffered channel can accept a range setpoint which may differ from the live (or C1) channel. For more information about the buffered channel, see the HP 3396 *Reference Manual*.

There will be an exclamation point after the C1 and buffer range values in the method listing when the HP 6890 is on the INET loop with the HP 3396. This indicates that the HP 6890 has accepted the range specified by the HP 3396.

Example

```
[LIST] [OP()] 8 [ENTER]

Range: C1, Buffer       4!4!
```

If you have an HP 6890 on the INET loop and the exclamation points are not present, the range parameter on the integrator is not tied to the range of the HP 6890. Reset the range in the OP() 8 dialog to force the HP 6890 to accept the values specified by the integrator. If the HP 6890 is not present on the INET loop, the exclamation points will never be present, and the OP() 8 dialog is not valid.

Storing and Loading a Method

Once you have created or made changes to a method, you can store it for future recall.

1. **Press** [**STORE**] [**METH**] *filespec* **[ENTER] to store a method.**

Example `* STORE METH A:GCMETH1 @`

stores the method as a file called GCMETH1.MET on external disk A. (If the extension .MET is not specified, the integrator appends it automatically.)

Once the integrator is switched off, the method is cleared. When the unit is powered-on, default method values will again be assigned. If a power failure occurs during storage of a method, a file error is returned and the method must be stored again.

Note: Any operation that involves a method file, such as storing a method, loading a method, or listing a remote method, requires that storage space be present on the M: disk. The space is used to store a temporary file while the file manipulation occurs. You will get an error message when you try to store a method to a disk with available space if there is no space on M:. The HP 3396 prints:

```
ATTEMPTED WRITE PAST END OF FILE.

DISK FULL.
```

This message could mean there is no storage space on the target disk, or on M:. If you get this message, first check the target disk for the file to be sure space is available. If there is enough storage space on the disk, obtain a directory listing of the M: disk to be sure there is some space available. If M: is full, copy some of the files to another disk, or delete some files, then continue.

2. **Press [LOAD]** [**METH**] *filespec* **[ENTER] to retrieve a stored method file.**

Caution **Loading a method from a file into the active workspace overwrites the active method including the entire Calibration Table. If you want to preserve it, save the current method in a file before loading a new one. Be careful when using this command.**

Example `* LOAD METH A:GCMETH1 @`

Any instrument on the INET loop may reject a method download. The following message is printed by the HP 3396.

```
INSTRUMENT AT ADDRESS 8 REJECTED SETPOINTS

METH NOT CHANGED
```

The address is the INET address of the instrument. You can list the stored method and the current active method to determine why the method was rejected.

The HP 6890 GC is designed to reject a method if the configuration does not match the current setpoints. For example, it will reject the method if the oven temperature maximum in the stored method differs from that in the active method, or if different detectors are installed on the GC. When a method is rejected by the HP 6890, an error message indicating why the method was rejected is printed by the integrator. An example message follows.

Example:

```
* [LOAD] [METH]  A: labmet1

INSTRUMENT AT ADDRESS 8 REJECTED PARAMETERS

Back Det Type Mismatch

ALS Back Tower

Oven Max Mismatch

Col Length
```

Sometimes the reason for the method rejection does not affect the current analysis. For instance, a different back detector may be installed from when the method was stored. If you are only using the front detector, the back detector mismatch is unimportant. Another frequent reason for a method mismatch is because the column length or diameter has changed. The HP 6890 uses the column information to calculate flow rates; the column flow will be different in the actual chromatography if the column dimensions in the stored method do not match those in the active method. The chromatographer can use the error messages to decide whether the GC should load the method anyway, or whether the active method should be modified before loading the stored method. The HP 3396 prompts the user with the option to force a best-fit method load.

Example:

```
*[LOAD] [METH]  A: labmet1

INSTRUMENT AT ADDRESS 8 REJECTED PARAMETERS

Back Det Type Mismatch

ALS Back Tower

Oven Max Mismatch

SHOULD ADDRESS 8 ADAPT PARAMETERS?   [Y/N*]
```

If you select Y, YES, the HP 6890 loads the method. The electrical parameters are changed. The configuration parameters are not. The method load is adapted to the current active method.

If you decide the reasons for the method load rejection are too important to accept a best-fit load, respond with N, NO. The integrator prints:

```
SHOULD ADDRESS 8 ADAPT PARAMETERS?   [Y/N*]

* N [ENTER]

METH NOT CHANGED
```

You can then modify the active method to better fit the stored method, or create a new method.

Editing a Method

Methods may be changed or new methods created by editing already existing method files.

1. **Load the method you wish to edit into the active workspace. (See instructions on the previous page.)**
2. **Press** [**EDIT**] [**METH**] **to edit the method in the active workspace.**

Example `* EDIT METH`

```
1 = RUN PARAMETERS
2 = TIMETABLE EVENTS
3 = CALIBRATION FILE
4 = INTEGRATION PLOT TYPE
5 = RUN DATA STORAGE OPTIONS
6 = REPORT OPTIONS
7 = POST-RUN LIST OPTIONS
8 = REMOTE DEVICE ACCESS

SECTION TO BE EDITED:
```

3. **Type the number that corresponds to the section of the method you wish to edit.**

```
SECTION TO BE EDITED:
```

will be repeated after exiting from each section in the edit dialog. To exit from a section of the edit dialog, use the **[ENTER]** key. The **[BREAK]** key quits the editing session completely and returns you to system command mode.

Once the material in the active workspace is edited, the revisions can be incorporated in the stored method by re-storing the method using the same method name.

The sections and parameters in the **[EDIT] [METH]** dialog duplicate the **[PREP] [METH]** dialog with the exception that the **[EDIT] [METH]** additionally allows you to send command strings to INET devices or a host computer through the `REMOTE DEVICE ACCESS` section of this dialog. Option 6 accesses the same dialog. See the HP 3396 *Reference Manual* for more information about option 6.

Listing a Method

1. **Press [LIST] [METH] [ENTER] to review the active workspace method.**

The integrator will print out the information entered for the active method:

- run parameters
- timetable events
- calibration
- integration plot mode
- run data storage
- report options
- post-run list options
- external event tables

The portions of the method stored in other INET instruments are also listed.

or

Press [LIST] [METH] *filespec* **[ENTER] to review a stored method.**

The integrator will look for a file with the .MET extension, even if you don't include one in the *filespec*. If the file does not exist or does not have a .MET extension, the unit will print `"FILE NOT FOUND"`. When you make copies (**COPY** command) of method files, be sure to add the .MET extension to the new file name.

The listing of a method from disk cannot be interrupted with the **[BREAK]** key.

Method List Options

HP 6890 method files may be too large for the buffer space in the HP 3396 Series II. (The HP 3396 Series III was designed to accommodate the method files of the HP 6890, so there is always enough buffer space.) Two method listing options are available to enable the HP 3396 Series II to handle HP 6890 method files.

1. Truncated Method

The HP 6890 delivers the method list information in 256 byte blocks. The HP 6890 can be instructed to truncate the method listing at the end of any block, from 1 to 255. This ensures that the buffer space in the HP 3396 Series II is not overfilled. The result of selecting a truncated method listing is that part of the HP 6890 method may not be listed as an off-line method. Since the latter part of the method listing shows detector configurations and other information that does not change very often, it doesn't usually matter if the entire method is listed.

2. Direct Listing

The direct list mode dumps the HP 6890 method directly onto the integrator paper, bypassing the buffer space entirely. It therefore doesn't matter whether the space is large enough for the method. A method stored while in Direct List mode cannot be listed from a disk; only the integrator portion of the method will be listed. To list the entire method, it must be reloaded into the active workspace.

To select the truncated or direct list modes, type:

***[OP()] 6 [ENTER]**

```
DEVICE ADDRESS:
```

8 [ENTER]

Select the INET address of your HP 6890 GC. The address is assigned by the integrator when the configured instruments are turned on. The GC is usually 8 or 9; to determine what the address of your GC is, use the

SYSTEM command. For more about INET addressing, see the HP 3396 *Reference Manual*.

COMMAND STRING: **LISTMETH DIRECT [ENTER]**

LISTMETH TRUNC [ENTER]

LISTMETH NORMAL [ENTER]

The LISTMETH command string selects the truncated or direct listing; LISTMETH NORMAL returns to the normal mode. The command must be typed in all capital letters.

If you have selected LISTMETH TRUNC, you select the size of the truncated method with the LISTSIZE command.

DEVICE ADDRESS: **8 [ENTER]**

COMMAND STRING: **LISTSIZE 125 [ENTER]**

The size can be any integer from 1 to 255. (Most GC methods are entirely listed by LISTSIZE 15; truncating at a larger LISTSIZE will ensure that the buffer space is not exceeded, but not have a visible effect on the method listing.)

To exit the dialog, just press [ENTER]

DEVICE ADDRESS: **[ENTER]**

The method list modes are retained by the HP 6890 until they are explicitly changed by the operator; they are not reset during a power failure or a cold start.

Example `* LIST METH A:GCMETH1 @`

```
RUN PARAMETERS
ZERO    = 0
ATT 2^  = 2
CHT SP  = 1.0
AR REJ  = 0
THRSH   = 2
PK WD   = 0.01
TIMETABLE EVENTS
0.300 PK WD   = 0.05
0.800 STOP
CALIBRATION
NORM
REF % RTW:  5.000  NON-REF % RTW:  5.000

LEVEL: 1                    RECALIBRATIONS: 1
CAL#  RT     LV        AMT        AMT/HEIGHT
1R    0.131   1    2.0000E+00    2.6159E-06
2     0.235   1    3.0000E+00    3.6887E-06
3R    0.298   1    4.0000E+00    4.9270E-06
4     0.506   1    5.0000E+00    7.1225E-03
5R    0.673   1    6.0000E+00    7.4056E-04
CAL#       NAME
  1       PK1
  2       PK2
  3       PK3
  4       PK4
  5       PK5
GRP#       CAL#            NAME
  1       2,  4,          GRP1
  2       3,  5,          GRP2
  3       4,  5,          GRP3
  4       2,  4,  5,      GRP4
  5       1,  3,  5,      GRP3
```

continued on next page

```
INTEGRATION PLOT TYPE ....... FILTERED
Presentation plot ........... NO

RUN DATA STORAGE

Store signal data ........... YES
Device ...................... M
Bunched or raw data ......... BUNCHED
Store processed peaks ....... NO

CALIBRATION OPTIONS

RF of uncalibrated peaks .... 1.0000E+00
Calibration fit ............. P
Disable post-run RT update .. NO
MUL FACTOR .................. 1.0000E+00

REPORT OPTIONS

Suppress local report ....... NO
Height% report .............. NO
Report title:
SLUDGE
Amount label ................ GMS
Report uncalibrated peaks ... YES
Extended report ............. YES

PRINT & POST-RUN LIST OPTIONS

Large font .................. YES
Store post-run report ....... NO
External post-run report .... NO
List run parameters ......... YES
List timetable .............. YES
List calibration table ...... NO
List remote method .......... NO
Form-feed before report ..... NO
Form-feed after report ...... NO
Skip perforations in report . NO
Skip perforations in plot ... NO

RANGE: C1,BUFFER............5!5!

19405B IECM CONTROL MODULE
 LOOP ADDRESS:  9
```

```
EXTERNAL EVENT TABLES
  NO AUTO RESET
TIME TABLE:
    TIME      EXT #     STATE
    0.24        3        ON
```

The GC portion of the method will be printed next.

Listing INET Instrument Set Points

1. **Press [LIST] [METH]** *device address* **[ENTER] to list the parameters for an INET GC.**

The loop address for an INET (Instrument Network) GC can be verified by pressing **[S][Y] [ENTER]**. See the HP 3396 Series *Reference Manual* for more information about INET.

Example `* LIST METH 8 @`

```
HP 5890A GAS CHROMATOGRAPH
 LOOP ADDRESS: 8

OVEN TEMP = 204    SETPT = 204
 EQUIB TIME   = 0.00
 CRYO OFF
 OVEN MAXIMUM = 400
 INITIAL TEMP = 204
 INITIAL TIME = 10.00

RUN LENGTH = 10.00 MIN

INJ A TEMP = 50   SETPT = 50
INJ B TEMP = 50   SETPT = 50
DET A TEMP = 203  SETPT = 203
DET B TEMP = 207  SETPT = 207

SIGNAL 1 = A
 INET FULL RANGE DATA ON
 RANGE = 0
 ZERO  = 0.0
 ATTN  = 4

SIGNAL 2 = B
 RANGE = 0
 ZERO  = -10429
 ATTN  = 10

DETECTOR A = FID  (ON)
DETECTOR B = TCD  (ON)   POLARITY =
+

PURGE A = OFF
PURGE B = ON
```

These parameters will also be listed with a method or with a report if you answer Y to the option 5 dialog question `"List remote method"`.

Deleting a Method

1. **Press [DEL] [METH]** *filespec* **[ENTER] to delete the active method, or a method file.**

Caution

When you use [DEL] [METH] without the filespec, you delete the entire method in the active workspace, resetting all operating parameters to their default values, including the entire Calibration Table. When you delete a method file with a filespec, you erase the file from the disk. Be careful when using this command.

Example

```
* DELETE  METH METH625.MET   @
```

deletes the specified method on the default disk. M: is the default disk unless another default has been specified; see chapter 7 for details.

Understanding Methods

An HP 3396 method is a collection of instrument operating parameters and data storage and report options that the integrator uses to conduct a run. A method may include a calibration table for determining how sample areas are to be converted for Normalization, ISTD (Internal Standard), ISTD%, ESTD (External Standard), or ESTD% reports. When Instrument Network (INET) instruments are connected to the integrator, the method includes their set points.

The active workspace always contains a complete set of operating parameters for making an analysis. Each individual method is composed of groups of parameters in the active workspace. Method parameters are accessible from the **[PREP]** or **[EDIT] [METH]** dialogs, the keyboard, or through the option dialogs.

Method parameters may be edited several different ways. The **[EDIT] [METH]** dialog allows selection of the appropriate entry group for editing. Certain method parameters may also be edited via the option dialogs 1 through 6. The **[EXT()]** and **[INTG()]** keys may be used for editing timetable entries, the **[PREP]** or **[EDIT] [CALIB]** dialogs for editing calibrations, and the run parameter keys on the keyboard. Regardless of how a change is entered, altering a method parameter alters the current method in the active workspace.

How to Access Method Parameter Groups

Method Parameter Group	Accessible directly from Keyboard	Accessible from Option Dialog
Run parameters	yes	—
Timed events	yes	—
Calibration parameters	no	**[OP()] [3]**
Integration plot type	no	**[OP()] [1]**
Run data storage options	no	**[OP()] [2]**
Report options	no	**[OP()] [4]**
Post-run list options	no	**[OP()] [5]**
INET instrument set points	yes	**[OP()] [6]**
Paper control parameters	no	**[OP()] [5]**
RANGE setpoints	no	**[OP()] [8]**

At turn-on, the active method consists of a set of default values. The listing of default method parameters shown on the next page is printed when no INET instruments are connected to the integrator.

Default Method Listing

```
*  LIST: METH  @

RUN PARAMETERS

ZERO   =  0
ATT 2^ =  0
CHT SP =  1.0
AR REJ =  0
THRSH  =  0
PK WD  =  0.04

TIMETABLE EVENTS
EMPTY

CALIBRATION
NO CALIB TBL

INTEGRATION PLOT TYPE ....... FILTERED
Presentation plot ........... NO

RUN DATA STORAGE

Store signal data ........... NO
Store processed peaks ....... NO

REPORT OPTIONS

Suppress local report ....... NO
HEIGHT% report .............. NO
Report uncalibrated peaks ... NO
Extended report ............. NO

PRINT & POST-RUN LIST OPTIONS

Large font .................. YES
Store post-run report ....... NO
External post-run report .... NO
List run parameters ......... NO
List timetable .............. NO
List calibration table ...... NO
List remote method .......... NO
Form-feed before report ..... NO
Form-feed after report ...... NO
Skip perforations in report . NO
Skip perforations in plot ... NO
RANGE: C1,BUFFER           5!5!
```

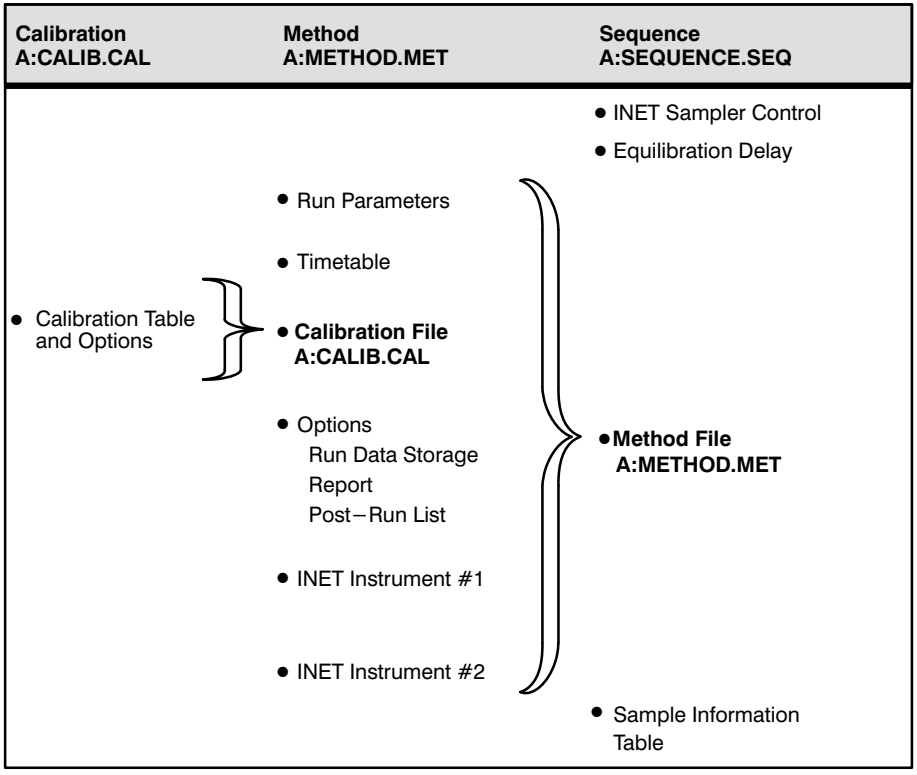
How Methods are Related to Sequences and Calibrations

Methods, automation sequences, and calibrations operate interdependently, although they are prepared separately using the **[PREP]** dialogs.

- Each calibration *must* be associated with a method.
- A method may *optionally* include a calibration.
- An automation sequence may name a method *or* use the currently active method parameters.

See chapter 5 and 6 for more information about calibrations and chapter 9 for more details about automation sequences.

Relationship Among Calibrations, Methods, and Sequences



Improving a Method with the Analyze Command

The **[PREP]** or **[EDIT] [METH]** and option 2 dialogs allow you to store signal data on the internal M: disk or on some external disk. Once signal data has been stored, it may be retrieved and re-analyzed in the future using the **ANALYZE** command.

Since new method parameters may be chosen before the reanalyzing takes place, the **ANALYZE** command allows method parameters to be improved based on actual run results. By changing parameters, reanalyzing the data, replotting with the baseline program, and printing a new report, a method may be effectively improved. After this fine-tuning process is completed, the updated method file must be stored for future use.

See the *Plotting a Baseline* and *Scheduling Postrun Programs* in *Using Applications Programs* for related information.

9

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Automating Analyses

Automating Analyses

What is a Sequence?

The HP 3396 automates an analytical system using sequences—sets of instructions that define how the integrator performs more than one automated run.

You can create sequences to run on INET (Hewlett-Packard's Instrument Network) or non-INET systems, with HP or non-HP samplers.

A sequence includes:

- sampler parameters (for some INET samplers)
- instrument equilibration time between analyses (up to 9.1 hours)
- a method (either stored or current)
- a sample information table (optional)

A sequence always references a method file or uses the active method parameters to define the GC or LC operation and integrator functions. Together, a method and a sequence determine how a series of analyses are carried out. See chapter 8 for a description of the relationship between methods and sequences.

Before You Prepare a Sequence....

1. **Determine which sequence control mechanism your instrument configuration employs.**

HP instruments can communicate with each other over the Hewlett-Packard Instrument Network, called INET. The first prompt in the sequence preparation dialog asks whether INET sampler control is being used. If an HP sampler is in use, the response to this prompt is *yes*, and you prepare an INET sequence. There are two types of INET sequences, described below. If you are using a non-HP sampler, INET is not used for instrument communication; the response to the first prompt is *no*, and you prepare a non-INET sequence.

- In a configuration of an HP 3396, HP 6890 GC, and HP 7673C Sampler, there is an RS232 connection between the sampler and GC. The GC controls the sampler over this connection. The ALS parameters are set at the keypad of the HP 6890. See the HP 6890 *Operating Manual* for detailed information on selecting the ALS parameters and preparing the sequence. The sequence content can be set at the keypad of the HP 6890, or during the [PREP][SEQ] dialog of the HP 3396. The HP 3396 integrator is in an INET configuration with the HP 6890, and receives the run or bottle number information from the HP 6890. The integrator method, calibration, and sample information table are prepared on the integrator. For the HP 3396 dialog that occurs in this configuration, see *Preparing an HP 6890/7673C/3396 Sequence*, below.
- In a configuration of an HP 3396, HP 5890 GC, and HP 7673 Sampler, all three instruments communicate in a loop over INET, with the integrator in control. The sequence preparation dialog on the HP 3396 includes the injection parameters and sequence content. It is described in *Preparing an INET Sequence with the HP 3396 in Control,* later in this chapter.

- A configuration in which a non-HP sampler is used is a Non-INET sequence. The sequence content and injection parameters are controlled by the sampler. The integrator treats the sequence like a series of manual runs. The integrator can receive bottle number information from the sampler if a bottle number cable is installed, or can identify the sequence by run number. See the dialog *Preparing a Non-INET Sequence* later in this chapter.

See the section of this chapter called *Understanding Automation* for more information about sequence control.

2. **Prepare the method and calibration table that you plan to use in your sequence.**

   Instructions for preparing a method are located in Chapter 8. Instructions for creating a calibration table are located in Chapters 5 and 6.

3. **Prepare the samples and position them in the automatic sampler.**

4. **Follow the instructions for preparing a sequence located below.**

   The [PREP] [SEQ] dialog provides a formal all-inclusive procedure for establishing active workspace values. Specific parameters may be set directly using [EDIT] [SEQ] or the Option 7 dialog.

Learning the Mechanics of the Sequence dialog

As you work through the sequence dialog, you will be asked to enter information and to make choices. When a selection of choices is given, one of the possible responses will be followed by an asterisk. The * marks the current selection. If the current selection is the one you want, press [ENTER]. If you want to change the current selection, enter the letter corresponding to the desired entry.

Example `INET SAMPLER CONTROL [Y/N*]`

N (No) is the current response and assumes you do not have an HP 7673 Automatic Sampler. Press [ENTER] to keep the response. See Preparing a Non-INET Sequence later in this chapter.

Y (Yes) If an HP 6890/7673C/3396 trio is present, go to *Preparing an HP 6890/7376C/3396 Sequence*, below. If an INET instrument group controlled by the HP 3396 is present, skip to *Preparing an INET Sequence with the HP 3396 in Control*, later in this chapter. Press Y [ENTER].

Example `BOTTLE OR RUN SAMPLE INDEXED [R/B*]:`

B Indexes a sequence by bottle number. This is the current selection; press [ENTER] to retain this response.

R Indexes a sequence by run number. Press R [ENTER] to change to this response.

The sequence dialog can be ended at any time with the [BREAK] key. All set points entered before the [BREAK] key are retained. All entries not reached before the [BREAK] key was pressed keep the values they had before the dialog began. After preparing the sequence you can store it; see Storing a Sequence, later in this chapter.

Preparing a Sequence

Preparing an HP6890/HP7673C/HP3396 Sequence

1. **Press [PREP] [SEQ] to start the dialog.**

```
*  [PREP] [SEQ]

ALS INFORMATION

INET SAMPLER CONTROL [Y/N*]   Y [ENTER]
```

2. **Press Y [ENTER] to indicate the presence of an HP 7673 sampler.**

The integrator now lists the INET 6890A GC, to which it is configured, with its appropriate loop address.

```
6890A GAS CHROMATOGRAPH:

          LOOP ADDRESS: 8

Sequence Type  (1= FRONT ALS, 2=BACK ALS, 3=BOTH ALS, 4=VALVE)
```

3. **Select the number for the automatic sampler you are using.**

If you pick option 1 or 2, indicating you will use either a front or back injector:

```
INJECTIONS PER VIAL:    1---->

FIRST BOTTLE NUMBER:    1---->

LAST BOTTLE NUMBER:     1---->
```

If you pick 3, indicating you will use dual injectors:

```
(FRONT) INJECTIONS PER VIAL:  1---->

(FRONT) FIRST BOTTLE NUMBER:  1---->

(FRONT) LAST BOTTLE NUMBER:   1---->
```

```
(BACK) INJECTIONS PER VIAL:   1---->

(BACK) FIRST BOTTLE NUMBER:   1---->

(BACK) LAST BOTTLE NUMBER:    1---->
```

Option 4 is usually used with gas sampling valves. If you select option 4 and no multiposition valve is installed:

```
# OF INJECTIONS:        1---->
```

If you pick option 4 and a multiposition valve is installed:

```
INJECTIONS PER POSITION:      1---->

POSITION RANGE:               1---->

TIMES THROUGH RANGE:    1---->
```

For all four injectors, the dialog continues:

```
Post Sequence GC Method #                 0---->

Repeat Sequence ?       1 = Yes, 0 = No   0---->

POSITION <1=FRONT, 2=REAR>

          INET CHANNEL 1       1 ------>  [ENTER]
```

The position indicates whether the HP 3396 is configured to accept data from the front or rear injector. The current configuration is the front injector; press [ENTER] to retain the front injector, and 2 [ENTER] to change to the back.

Note: The parameters in step 3 can also be set at the keypad of the HP 6890. If you set them there, you do not need to also set them in the sequence preparation dialog. They will appear as the default values in the dialog above; just press [ENTER] at each prompt to retain them. There is only one set of sequence parameters in the active workspace at any time, whether it is selected in the sequence dialog of the HP 3396, at the keypad of the HP 6890, or loaded from a stored sequence file.

```
EQUILIBRATION TIME IN SECONDS [0]
```

4. **Fill in an equilibration time, if desired.**

The equilibration time will allow extra time for the GC oven temperature to equilibrate at the initial set point before each injection.

Sample Information Table

The last several lines of dialog allow you to customize, by way of a Sample Information Table, how each sample or run is calculated. The sample table is listed when you list the sequence. For related infomation see the *Sample Information Table* section later in this chapter.

```
Delete Sample Table [Y/N*]:
```

5. **Type Y to create a new sample table.**

This prompt is printed only if a sample table already exists. Pressing **N** keeps the old sample table.

```
BOTTLE OR RUN SAMPLE INDEXED [R/B*]:
```

6. **Type R to make the run number the index into the Sample Information Table or press [ENTER] to the bottle number as the Sample Table index.**

If you select B, bottle number indexing, the sequence will ignore the run number on the HP 3396 and use the injection parameters set on the HP 6890. If you select R, run numer indexing, the sequence will not execute if the current run number on the HP 3396 is greater than the number of the bottle position of the HP 7673. It is best to select bottle number indexing when the HP 3396 is configured with an HP 6890.

If you answered **Y** to `INET SAMPLER CONTROL [Y*/N]:` above, the dialog skips to step 9.

```
FIRST BOTTLE  [1    ]:
      or
FIRST RUN [1    ]:        (an integer from 0 to 32767)
```

7. **Enter the first run or bottle number to be indexed.**

```
LAST BOTTLE  [1    ]:
      or
LAST RUN [1     ]:              (an integer from 0 to 32767)
```

8. **Enter the last indexed run or bottle number.**

```
BOTTLE  # :  or RUN  # :         (an integer from 0 to 32767)
```

9. **Enter the number of the first bottle or run number to be included in the table.**

```
ISTD  AMT:                  (default = 0000.00)
```

10. **Enter the amount of internal standard added to the sample.**

If you enter a SAMPLE AMT (see step 25 below), the calculation will be in percent-of-sample units.

```
SAMPLE AMT:                 (default = 0000.00)
```

11. **Enter a sample amount if you want an ISTD% or ESTD% calculation.**

```
MUL FACTOR:                 (default value = 1.0)
```

12. **Enter the desired multiplication factor.**

This parameter corrects for dilution, unit conversions, etc.

RECALIBRATION [Y/N*]:

13. **Type Y to recompute response factors and retention times based on this (standard) sample, or press [ENTER] if no recalibration is desired.**

If recalibration is not requested, the dialog skips to step 29.

LEVEL: *(optional [−] and an integer from 1 to 63)*
(default value = 0)

14. **Enter the level you wish to recalibrate.**

For a description of levels of recalibration and when it is legal to enter a level higher than 1, see chapter 4.

Preceding the level with a **[−]** will replace the old calibration data with new. Without the **[−]**, the old and new data are averaged.

NAME *(1 to 8 characters)*

15. **Enter a name for this bottle or run number.**

Starting with a letter, you may create a sample name with letters and numbers up to eight characters in length.

REPORT MEMO *(1 to 126 characters)*

16. **Type any Sample Information Table notations you wish printed in the report.**

BOTTLE #: *or* RUN #:

17. **Continue to enter sample information until the table is complete. Press [ENTER] key without entering a bottle or run number to end the sample table dialog.**

This dialog repeats itself to create a sample table with as many as 32767 entries.

```
NEXT SEQUENCE:[*]
```

18. **Enter the name of a sequence file if you want another sequence to execute after the current sequence.**

The NEXT SEQUENCE prompt allows you to chain sequences. Each sequence in the chain will call another sequence when it is finished. Each sequence can have a unique method and injection parameters, and you can chain them together indefinitely. If the sequence file does not exit, the HP 3396 prints:

```
Caution:  Cannot get sequence file.
```

The current sequence will execute, but the NEXT SEQUENCE function will fail if the sequence file does not exist.

You can also chain sequences using the Sequence Chain Applications Program; see the *Using Application Programs* Manual for more information.

About the Sample Information Table

The last several lines of the [**PREP**] [**SEQ]** dialog allow you to customize, by way of a sample information table, how each sample or run is calculated. You may choose to organize the table by bottle or run numbers.

Information from the sample table is used in the report when a bottle or run number in the table matches the current bottle in the sampler or the current HP 3396 run number. The report will then include the appropriate sample numbers, names, and report memos, etc from the sample information table. (The `MUL FACTOR` and `SAMPLE AMT` may also be entered via option 3.)

After at least one bottle or run number has matched an entry in the table, any subsequent bottle or run number that doesn't match will use the sample values from the previous run or bottle. This allows consecutive samples to be handled identically without having to make separate entries in the sample table for each one.

If there is no sample information table or if bottle or run numbers don't match the sample table at the start of a sequence, option 7 values are used until a match is found. Note that the bottle numbers entered in this dialog *must* match the incoming BCD (binary-coded decimal) numbers to successfully automate non-INET sampling equipment. When BCD input is outside the range of `FIRST BOTTLE #`/`LAST BOTTLE #`, the HP 3396 will print `INVALID SAMPLE NUMBER`.

The sample table is listed when you list a sequence or option 7.

Example `* LIST: OP # 7`

```
DEFAULT SAMPLE INFORMATION
USE SAMPLE TABLE IN MANUAL RUN ..... NO

ISTD AMT . 0.0000E+00
SAMPLE AMT0.0000E+00
MUL FACTOR   . 1.0000E+00
RECALIBRATION  NO
```

Default values for option 7 are listed in the example above and do not include a sample name or report memo.

Preparing an INET Sequence with the HP 3396 in Control

If you are using any INET instrument group which does not include the HP 6890 GC, the injection parameters are set in the HP 3396 sequence preparation dialog. After setting up the injection parameters, the dialog continues with EQUILIBRATION TIME IN SECONDS, and is identical to the dialog described in *Preparing an HP 6890/HP 7673/HP 3396C Sequence*.

The integrator controls the sequence of injections from the HP 7673 Automatic Sampler. If an HP 7673 is not equipped with a tray, only bottle numbers 1−3 are valid `FIRST BOTTLE #`, `LAST BOTTLE #` entries. When a bottle number greater than 3 is entered for the FIRST BOTTLE #, the sequence will be aborted before any injection takes place, and the unit will print an error message similar to the one printed below.

```
7673A SAMPLER*

LOOP ADDRESS: 9

INVALID BOTTLE NUMBER
```

If, however, a number less than or equal to 3 is entered for `FIRST BOTTLE #` and a number greater than three is entered for the `LAST BOTTLE #`, the sampler will inject as many bottles as it can up to three and ignore the incorrectly set `LAST BOTTLE #`. No error message is printed in this case. Refer to the *HP 7673 Operation and Service* Manual for more details.

The HP 7673 may be programmed using BASIC commands. The **INET_IO** command allows device-dependent commands to be sent to the INET sampler. See the **INET_IO Commands** in the *HP 3396 BASIC Language Reference* Manual for more details.

1. **Press [PREP] [SEQ] to begin the sequence preparation dialog.**

* [PREP] [SEQ]

```
INET SAMPLE CONTROL [Y/N*]:
```
Y [ENTER]

2. **Press Y and [ENTER] to indicate an INET configuration.**

```
5890 SERIES II GAS CHROMATOGRAPH

   LOOP ADDRESS:  8

7673C AUTOMATIC SAMPLER

   LOOP ADDRESS:  9
```

The loop address is a number from 8 to 31 automatically set by the integrator when it configures the INET loop. See the HP 3396 *Reference Manual* for more information on INET addressing.

The dialog begins by setting up the injection parameters for the HP 7673. The HP 7673 inserts its own preset dialog into the HP 3396. For additional information on the HP 7673 dialogs, see the HP 7673 Automatic Sampler *Operating Manual*.

```
ENABLE TRAY (1=YES, 2=NO)     1-->

ENABLE BAR CODE               1-->

FRONT INJECTOR

   INJ/BOTTLE              1-->

   FIRST BOTTLE            1-->

   LAST BOTTLE             1-->

   # OF SAMPLE WASHES            0-->

   # OF PUMPS              6-->

   VISCOSITY               0-->

   VOLUME                  1-->

   # OF SOLVENT A WASHES         0-->

   # OF SOLVENT B WASHES   0-->
```

```
   PRIORITY SAMPLE (1=YES) -->
   CAPILLARY ON-COLUMN     0-->
REAR INJECTOR
   INJ/BOTTLE     1-->
   FIRST BOTTLE   1-->
   LAST BOTTLE    1-->
   # OF SAMPLE WASHES      0-->
   # OF PUMPS     6-->
   VISCOSITY      0-->
   VOLUME         1-->
   # OF SOLVENT A WASHES   0-->
   # OF SOLVENT B WASHES   0-->
   PRIORITY SAMPLE (1=YES) -->
   CAPILLARY ON-COLUMN     0-->
POSITION (1=FRONT, 2=REAR)
   INET CHANNEL 1          1-->
   INET CHANNEL 2          1-->
   AUXILIARY CHANNEL       1-->
EQUILIBRATION TIME IN SECONDS:
```

From this prompt on, the dialog is the same as the HP 6890/HP 7673/HP3396 dialog.

Preparing a Non-INET Sequence

You can automate analyses with non-INET equipment, made by HP or other manufacturers, by coordinating a series of manual runs. In this system, the integrator sends out a START signal over the remote control cable after receiving READY signals from the GC and sampler. Each run appears as a manual run to the HP 3396. The non-HP sampler controls the sequence and injection parameters.

The sequencer is the controller in this system. The controller must be capable of operating the sampler and be electrically and logically compatible with both the GC and integrator START inputs. An external sampler, an external valve, or another instrument is synchronized to the START run signal of the integrator via the Remote Start cable *or* the START RUN_ NOW, START RUN_LATER statements in BASIC.

For the non-INET sequence, the indexing mode is coupled with the run automation. Any binary-coded decimal (BCD) digits the sequencer supplies to the integrator are printed as the “SAMPLE #” for each bottle or run. In this way, the system can be set up for a series of injections, with the reports reflecting appropriate run or bottle numbers.

See *Listing a Sequence* for an example listing of a non-INET sequence.

Default Sequence Parameters for Non-INET Equipment

Parameter	Default Value	Permitted Range
INET Sampler Control	N	Y, N
Runs Wait for Start_Input*	No	Y or N
Remote_Start_Relay: Prerun/Run**	Run	P or R
Equilibration Time	0	0−32767
Remote Ready Sensing***	S	C or S
Method	unspecified	8 characters (First character must be a letter.)
SAMPLE INFORMATION TABLE		
Bottle or Run Sample Indexed	B	R, B
Bottle # or Run #	unspecified	0−32767
ISTD Amt	0000.00	
Sample Amt	0000.00	
Mul Factor	1.0	
Recalibration	N	Y, N
Level	1	1−63
Name	unspecified	8 characters
Report Memo	unspecified	126 characters
Next Sequence	unspecified	8 characters

*This prompt occurs only when a non-INET sequence is selected.
**This prompt occurs only when "Runs Wait for Start_Input", above, is YES.
***This prompt occurs only when equilibration time is not zero.

Automating by BOTTLE

In order to automate by bottle number, a bottle number cable (BCD) must be installed between the non-INET sampler and the HP 3396; this cable connects to the SAMPLE receptacle on the back of the integrator. In addition, a remote control cable (RSS) must be installed. See the HP 3396 Networking Guide for more information. If bottle numbers are not supplied through the cable in the HP 3396 SAMPLE receptacle, run number indexing should be chosen. The bottle numbers listed on the integrator must match the bottle numbers supplied over the BCD cable.

1. **Program the non-INET sampler or sequence programming device.**
2. **Make sure the sampler and the integrator are connected via the sample number (BCD) and remote control (RSS) cables.**

See the HP 3396 *Reference Manual* for information about how to install these cables.

3. **Press [PREP] [SEQ] to begin the sequence preparation dialog.**

```
*PREP SEQ

ALS INFORMATION
INET SAMPLER CONTROL [Y/N*]:
```

4. **Type N and press [ENTER] to indicate a non-INET sequence.**

Without INET sampler control, the integrator assumes either it will control the sequence by run number or that the runs will be started manually or by a remote control cable. To the HP 3396, this appears the same as a series of manual runs.

```
RUNS WAIT FOR START_INPUT: [Y/N*]

REMOTE_START_RELAY:  PRERUN/RUN [P/R*]
```

5. **Select the START mode you desire.**

If you select RUNS WAIT FOR START_INPUT: N (NO), the integrator will generate a pulse that starts the run. If you select Y (YES), the integrator will wait for a pulse generated by an external device (in this case, the sampler) to start the run.

The REMOTE_START_RELAY: PRERUN/RUN prompt occurs only when the RUNS WAIT FOR START_INPUT: [Y] mode is selected. If you select REMOTE_START_RELAY: PRERUN, the integrator will start the autosampler with a contact closure via SO1/SO2, without starting the run. The sampler prepares to inject, then starts the run when it injects the sample. If you select REMOTE_START_RELAY: RUN, the integrator starts the run without waiting for the sampler to prepare to inject via a contact closure on SO1/SO2.

This section of the dialog allows the user to coordinate the run start time to the injection cycle of the non-INET sampler. The RUNS WAIT FOR START_INPUT: [Y] mode and REMOTE_START_RELAY: PRERUN mode are functional only if the RSS cable you use is properly configured to your GC. The general purpose RSS cable recommended for this purpose is HP part # 03394-60540. This cable is described in the HP 3396 Series III *Reference Manual*.

```
EQUILIBRATION TIME IN SECONDS [0]
```

6. **Fill in an equilibration time, if desired.**

The equilibration time will allow extra time for the GC oven temperature to equilibrate at the initial set point before each injection. If the equilibration time is zero, the dialog skips to number 8.

```
REMOTE READY SENSING [C/S*]
```

7. **Indicate whether the remote ready sensing wires are connected or separated.**

This prompt only occurs with a non-zero equilibration time, in a non-INET sequence.

The HP 3396 needs to know if the remote ready sensing wires in your remote start stop (RSS) cable are tied together to correctly manage the equilibration time. If the remote ready sensing wires are tied together, respond C, connected; if they are separate, respond S.

To respond appropriately to this prompt, you must know what RSS cable you are using.

If you are configured to any analytical instrument with a general purpose (spade lug) cable, (such as HP part# 03394-60540), or with a cable you fabricated, examine the wires to see if the remote ready sensing wires are connected.

If you are using the RSS cable HP part # 03394-60600, the remote ready sensing wires are connected.

If the HP 3396 Series III is configured to the HP 6890 in a non-INET mode, you could use HP part # 03396-61010 instead of a general purpose cable. This special cable is designed to take advantage of the sequence capabilities of the HP 6890; the HP 6890 starts and stops the sequence, and the sequence is controlled on the keyboard of the HP 6890. The integrator therefore ignores the remote ready sensing parameter.

If you respond S and the HP 3396 fails to become ready for the second and subsequent injections in a sequence after a reasonable wait for the equilibration time, the wires in your cable are probably connected. Change the response to C.

8. **Create a Sample Information table indexed by `BOTTLE #` using the [PREP] or [EDIT] [SEQ] dialog.**

Instructions are located in the previous section of this chapter.

9. **Press [OP()] [7] [ENTER].**

```
USE SAMPLE TABLE IN MANUAL RUN [Y/N*]:
```

10. **Press Y to use the sample table.**

You must select Y, since the sequence appears to the integrator like a series of manual runs.

11. **Program a stop time for the runs.**

Example [TIME] [10.5] [STOP]

12. **Start the manual run with a signal from the non-INET sampler via the RSS cable, or by pressing START on the HP 3396.**

Do *not* press [SEQ] [START], because the integrator is expecting a manual run.

Automating by RUN

In order to automate by run number, the integrator run number must be reset before the sequence starts to match the run numbers listed in the Sample Information table.

1. **Program the non-INET sampler or sequence programming device.**
2. **Make sure the sampler and the integrator are connected via the remote control cable.**

See the HP 3396 *Reference Manual* for information about how to install this cable.

3. **Press [PREP] [SEQ] to begin the sequence preparation dialog.**

```
*PREP SEQ

ALS INFORMATION
INET SAMPLER CONTROL [Y/N*]:
```

4. **Type N and press [ENTER] to indicate a non-INET sequence.**

Without INET sampler control, the integrator assumes it will control the sequence, or that the runs will be started manually or by a remote control cable.

5. **Create a Sample Information table indexed by `RUN #` using the [PREP] or [EDIT] [SEQ] dialogs.**

`FIRST RUN #` and `LAST RUN #` define the bounds for a sequence of runs. Instructions are located in the previous section of this chapter.

6. **Press [OP()] [7] [ENTER].**

```
USE SAMPLE TABLE IN MANUAL RUN [Y/N*]:
```

7. **Press Y to use the sample table.**

8. **Program a stop time for the runs.**

Example [TIME] [10.5] [STOP]

9. **Set the run number to the value of `FIRST RUN #`, previously entered in the [PREP] or [EDIT] [SEQ] dialog.**

Example `* EDIT SEQ`

```
1 = ALS INFORMATION
2 = EQUILIBRATION TIME DELAY
3 = METHOD FILE SPECIFICATION
4 = SAMPLE INFORMATION TABLE
5 = NEXT SEQUENCE

SECTION TO BE EDITED:4 [ENTER]

SAMPLE INFORMATION TABLE
BOTTLE OR RUN NUMBER INDEXED [R/B*]:R [ENTER]
FIRST RUN [1    ]:5 [ENTER]
LAST RUN [1     ]:10 [ENTER]
```

the dialog continues . . .

```
*  OP  #  7

DEFAULT SAMPLE INFORMATION
USE SAMPLE TABLE IN MANUAL RUN [Y/N*]: Y [ENTER]

ISTD AMT [1.0000E+01  ]: [BREAK]

*SET RUNNUM 5  @
```

This example shows how to choose run number indexing and reset the run number to match the initial entry in the sample information table.

10. **Start the manual run with a signal from the non-INET sampler via the remote control cable.**

Do *not* press [**SEQ]** [**START**] to begin a manual run.

The remote control cable will hold the integrator off from starting any run in the sequence until the sampler is ready. An equilibration time may also be entered in the **[PREP]** or **[EDIT] [SEQ]** dialog to pause the system between runs.

Caution **When automating analyses with non-INET equipment, the sampler may take additional time before it makes an injection. This additional time may add significant and possibly nonreproducible delays in retention times and signal computation.**

To avoid this problem, select the RUNS WAIT FOR START_INPUT: [Y] mode and REMOTE_START_RELAY: PRERUN in the [PREP][SEQ] dialog. These modes are explained on page 9−16. They are functional only if the RSS cable you use will generate a pulse on pin 3, and is properly configured to your GC. The general purpose RSS cable recommended for this purpose is HP part # 03394-60540. This cable is described in the HP 3396 Series III *Reference Manual*.

Starting a Sequence

1. **If you are using INET instruments check that all INET instruments are connected and configured properly.**

Any **[START]** key on an INET instrument may begin a sequence by using the following one-line BASIC program:

```
10   START SEQ_LATER
```

This program delays the start of a sequence until an INET device start key is pressed.

2. **Type [S] [Y] [ENTER] and [R] [E] [A] [ENTER] to check the configuration and readiness of external devices for storage and retrieval of files.**

3. **Press [SEQ] [START] to begin a sequence.**

The automated sequence starts with the `FIRST BOTTLE #` or `RUN #` specified and will continue the sequence through the `LAST BOTTLE #` or `RUN #`. If the current run number is not within the `FIRST RUN #` and `LAST RUN #` range, and a sequence has already been started, the integrator prints `INVALID INDEX; ABORTED.`

If an instrument is not ready when the sequence is started, the integrator prints

```
WAITING FOR SYSTEM READINESS
```

or

```
EQUILIBRATION DELAY IN PROGRESS
```

Since the method file is not loaded into the active workspace until the sequence is started, a sequence with an invalid method will be aborted at the time the sequence is started. The integrator will print

```
FILE NOT FOUND
```

filespec `NOT LOADED`

A sequence will also be aborted before it is started if the disk indicated for data storage is not operational. An error message `DISC DOES NOT EXIST` or `HPIL DOWN` is printed.

A sequence will not begin if the method stores data to a disk that is full, and the sequence stops if the disk that is storing data becomes full during the sequence. The HP 3396 prints:

```
DISK FULL.

PRESS I, S, R, STOP TO IGNORE, SUSPEND, RESUME, OR STOP
```

If you press I, the sequence continues without storing the data. The HP 3396 prints an error message at the beginning of each run in the sequence, warning that the data is not stored.

S suspends disk checking so that, if a floppy disk is the target, the disk can be removed from the drive and another disk inserted without damage to the disk or drive.

R then resumes disk checking, and automation will continue if the new disk has space available.

STOP aborts the sequence. The integrator can then be turned off and the expansion card changed. The sequence must be manually started after the new card is inserted and the integrator is turned back on.

You can turn off the HP 3396 without responding to the prompt, change the expansion card, and turn the integrator back on. The HP 3396 will respond as to a power failure; the sequence will automatically resume at the bottle number where it stopped.

Note: Key entries must be in upper case. The HP 3396 silently ignores lower case entries.

Stopping a Sequence

1. **Press [STOP] while a sequence is in progress to cancel the sequence and the current run immediately.**

An INET automated sequence can be aborted by pressing the **[STOP]** key on the integrator, the INET sampler, or the INET GC. Sequence parameters in the active workspace of the HP 3396 remain unchanged. The integrator prints ABORTED and waits for further input. If an HP 6890 with a post-run program is in use, the post-run program will execute before the HP 3396 becomes ready for input. To stop the post-run program, press STOP on the HP 6890.

If the INET loop is broken or the power fails during a sequence, the run will stop immediately. This premature stop may result in an incomplete run.

The report reflects whatever data was collected during the run. When the loop recovers, the sampler will inject the previous sample (the one running when the loop was broken), and continue with the sequence. If the loop is broken or the power fails during a sequence, but while a run is not in progress (for instance, during a post-run program) the sampler will not repeat the previous injection, since the data was already obtained. Do not press STOP while the loop is down, or the sequence will abort.

If you are operating a non-INET sequence with the HP 6890, there are two STOP functions available. If you are using cable part # 03396-61010, pressing [STOP] on the HP 3396 stops the current run and aborts the sequence. Pressing STOP on the HP 6890 stops the current run, but the sequence continues as soon as the GC is ready. If you do not use this cable (either because you are using INET or a different RSS cable), pressing either stop key stops the run and aborts the sequence. See the HP 3396 *Reference Manual* for more information about RSS cables.

Storing and Loading a Sequence

Only one sequence can exist in the active workspace at any one time. To create a second sequence without losing the first one, you must store the original sequence as a .SEQ file.

Caution **Be sure that the storage device you intend to send a file to is properly linked to the HP 3396 before attempting to use the [STORE] function.**

1. **Press [STORE] [SEQ] *filespec* [ENTER] to store a sequence.**

When you store a sequence, you must name it so that you will be able to retrieve it. Making a note of the names of your sequence files, or using the directory command (refer to chapter 7 of this manual) will help you retrieve a particular sequence file at a later date. The HP 3396 adds a .SEQ extension to the file name you choose, if you don't include one.

2. **Press [LOAD] [SEQ] *filespec* [ENTER] to retrieve a sequence.**

The file must have a .SEQ extension to be loaded. The integrator will look for .SEQ extension even if you do not specify it in the *filespec*. The message

```
FILE NOT FOUND
SEQ NOT CHANGED
```

is printed if you specify a sequence file that does not end in .SEQ.

In any case where the sequence file cannot be loaded, the unit prints an appropriate error message and follows it with the `SEQ NOT CHANGED` message or the `DEFAULT SEQ INVOKED` message. Other messages may follow.

As with methods and calibration tables, only one version of a sequence can exist in the active workspace at any one time. If sequence files are to be saved and used again, they must be stored (via the **[STORE]** function) to an external disk. Otherwise, once the HP 3396 is switched off, the current sequence, including the sample information table, is erased.

Editing a Sequence

You may change one or more sequence parameters via the **[EDIT] [SEQ]** dialog. If you plan to use the original version of the sequence again in the future, be sure to store it before attempting to make any changes. Use the **[STORE]** command to save a sequence for future use.

A sequence file already stored must be downloaded from the storage device to the HP 3396 active workspace before you enter the [**EDIT] [SEQ]** dialog.

1. **Press [EDIT] [SEQ] [ENTER] to edit a sequence in the active workspace.**

```
1 = ALS INFORMATION
2 = EQUILIBRATION TIME DELAY
3 = METHOD FILE SPECIFICATION
4 = SAMPLE INFORMATION TABLE
5 = NEXT SEQUENCE

SECTION TO BE EDITED:
```

2. **Type the appropriate number to access the part of the sequence you are interested in changing.**

Enter **1** to change the sampler control from INET to non-INET and modify the sampler parameters.

Enter **2** to change the equilibration time and **3** to change the method file. To remove any reference to a method file , enter a space [ ] and then press **[ENTER]** at the method prompt. The previous method name will be erased.

Enter **4** to alter the Sample Information Table. To change the sample table, entries must be overwritten or deleted. Any entries not written over will remain as is. For example, if only the index (`BOTTLE #` or `RUN #`) is changed, the rest of the sample information will remain the same.

To delete sample information without writing over it, enter the bottle or run number to be deleted with a minus sign in front of it.

Example `RUN  #:` **−1**

The minus sign indicates that the first run in the sample table will be deleted.

3. **Use the [STORE] function to save an edited sequence for future use.**

Listing a Sequence

1. **Press [LIST] [SEQ] [ENTER] to review the sequence in the active workspace.**
2. **Press [LIST] [SEQ] *filespec* [ENTER] to review a stored sequence.**

The integrator will look for a file with a .SEQ extension if you don't include one in the *filespec*. If the file does not exist or does not have a .SEQ extension, the unit will print "FILE NOT FOUND". When you make copies of sequence files be sure to add the .SEQ extension.

Example `* LIST: SEQ AUTO1.SEQ @`

```
ALS INFORMATION
INET SAMPLER CONTROL ............... NO

RUNS WAIT FOR START_INPUT: [Y/N]     Y
REMOTE_START_RELAY: PRERUN/RUN [P/R*]....P

EQUILIBRATION TIME IN SECONDS ... 5

REMOTE READY SENSING ..........SEPARATE

METHOD ..................... METH1.MET

SAMPLE INFORMATION TABLE
BOTTLE OR RUN SAMPLE INDEXED .... R
FIRST RUN ....................... 5
LAST RUN ........................ 10
```

```
RUN # ..................... 5
ISTD AMT .................. 0.0000E+00
SAMPLE AMT ................ 6.2500E+00
MUL FACTOR ................ 3.1700E+00
RECALIBRATION ............. NO
NAME ...................... GROUP A
```

the sample table continues for runs 6 through 10...

```
NEXT SEQUENCE ............. NEXT.SEQ
```

Deleting a Sequence

Caution

Be sure you want to delete the sequence before you execute the keystrokes. If you omit filespec, the sequence in the active workspace will be deleted—that is, returned to its default values. Be sure you have stored a sequence you want to save for future use BEFORE you delete it from the active workspace or overwrite it by preparing a new sequence.

1. **Press [DEL] [SEQ] *filespec* [ENTER] to delete any sequence.**

When you delete the sequence in the active workspace (no *filespec* indicated), the default parameters are in effect until a new sequence is prepared.

Linking Sequences

Sequences may be linked using the NEXT SEQUENCE prompt in the [PREP][SEQ] or [EDIT][SEQ] dialogs, or with the Sequence Chain Applications Program. Refer to *Using Applications Programs* for information about the Sequence Chain Applications Program.

HP 19405A S/ECM and HP 19405B IECM Operation

Existing HP 19405A Sampler/Event Control Modules can be used with the HP 3396 Series III exactly as they are with the HP 3396 Series II; see your HP 19405A *Operating Manual* for more information.

The HP 19405B INTEG Event Control Module (IECM) operates with the HP 3396 integrator to control external events in INET or RS232 configurations. The HP IECM supports eight 250V relay connections for external device control. Unlike the HP 19405A S/ECM, the IECM does not support sampler control.

The IECM has two LEDs on the front panel. One indicates a normal powered-up condition when lit, and the other flashes to show an event is in progress.

As an INET device, the HP IECM produces sample and annotation data, and accepts keyed-in set points. The HP 3396 provides an ON/OFF switching signal to the eight event-control relay outputs through the [EXT()] key. HP 19405B external events can also be turned ON and OFF from the HP 3396 via the SET EXT BASIC command. See the HP 3396 Basic Language manuals for more information.

An External Event occurs when the HP IECM switches through a relay output. External Events may be executed immediately or listed in their own timetable and stored as part of the method.

WARNING **Relay Switches 1 through 8 may have high voltage levels associated with them, depending upon the specific installation. For safety, treat them as if they were connected to their full-rated voltage (250 V ac and 5 A maximum). Failure to do so may result in injury or death, as well as damage to the instruments.**

Executing EXT() Commands

Manual switching of external events may be done before or during a chromatographic run. When an event is switched during a run, "EX" is printed on the chromatogram.

1. **Press [EXT()] *event number* [ENTER] to switch a channel ON immediately.**

The *event number* is an integer from 1 to 8 and is determined by which IECM output the device is connected to.

2. **Press [EXT()] [−] *event number* [ENTER] to switch a channel OFF immediately.**

Time-Programming EXT() Commands

You may time-program a maximum of thirty-five external events. During a power failure, the integrator can save the first twenty events.

1. **Press [TIME] *run time* [EXT()] *event number* [ENTER] to enter an external event into the timetable .**

run time is the time during the run when the event will happen. The *event number* is an integer from 1 to 8 and is determined by which IECM output (*event number*) the device is connected to.

2. **Press [DEL] [TIME] *run time* [EXT()] *event number* [ENTER] to delete individual events.**

   Press [DEL] [TIME] [EXT()] [ENTER] to delete the entire external events timetable.

Using Auto Reset

The auto reset state is the state to which the external event returns immediately following a run. When the auto reset is ON, events return to the state they were in at the beginning of the run. Auto reset is OFF unless an equilibration time is entered.

1. **Set all external events to the state at which you want them to reset at the end of the run.**
2. **Press [TIME] *equilibration time* [EXT()] [ENTER] to set a time for programmed external events to equilibrate before the next run.**

The *equilibration time* is the time from the end of the run to when the IECM becomes ready on INET.

3. **Press [TIME] [0] [EXT()] [ENTER] to disable auto reset and the equilibration time.**

Listing External Events

1. **Press [LIST] [TIME] [EXT()] [ENTER] to print the external event timetable.**

Example

```
*   LIST: TIME   EXT #   @
19405B EVENT CONTROL MODULE
   LOOP ADDRESS:   9

 EXTERNAL EVENT TABLES

    AUTO RESET:
   EXT #   STATE
   -----   -----
     1       OFF
     2       OFF
     3       OFF
     4       OFF
     5       OFF
     6       OFF
     7       OFF
     8       OFF
   EQUILIBRATION TIME         0.10

TIME TABLE
   TIME    EXT #   STATE
   ----    -----   -----
   0.00       1     OFF
   1.55       2     OFF
   3.26       3     ON
   5.00       4     ON
   6.00       5     OFF
   6.00       6     OFF
   6.00       7     OFF
   6.00       8     OFF
```

2. **Press [LIST] [EXT()] {*event number*} [ENTER] to print only the current status of an external event.**

If *event number* is omitted, the current status of all eight channels is listed.

Example To set the auto reset state to **OFF** for all External Events at an equilibration time of 0.1 minute, first turn OFF each external event that is not already OFF.

```
*  EXT #  -3  @
*  EXT #  -4  @
```

Then set an equilibration time of 0.1 minute to enable the auto reset.

```
*  TIME  .1  EXT #  @
```

See the example under *Listing External Events* to see the auto reset and timetables that are created from this example.

HP 19405B IECM Status

Two status indicators on the front panel of the HP 19405B IECM show the various modes of operation. For information about INET states, see the HP 3396 *Reference Manual*.

HP 19405B Power-Fail Backup and Recovery

The integrator allocates space in its local storage to backup IECM set points. The first 20 external events in the timetable are preserved during a power failure of less than 95 hours. A longer power failure may result in loss of all data stored in the integrator. For protection from power failures, the external event table should be stored for reuse on a disk.

10

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Using Reports

Using Reports

Getting Default Reports

Data analyzed by the integrator from a real-time run or reintegration is consolidated in a printed report. The simplest report is the AREA% report, produced by default.

1. **Press [AREA%] to produce an AREA% report from the last real-time run or reintegration.**

If HEIGHT% was selected in option 4, a HEIGHT% report is printed when **[AREA%]** is pressed. (The option 4 dialog is discussed later in this chapter.)

Example

```
          * AREA%

          RUN# 65          OCT  5, 1994  09:01:32

          SAMPLE# 7

          SIGNAL FILE: M:SIGNAL.BNC

          TECH PROPIONIC ACID

AREA%

       RT         AREA     TYPE       WIDTH          AREA%

     .138         5076       PP         .015         .03506

     .200        57910       PB         .021         .40000

     .295       487595       PB         .022        3.36799

    2.220        32238      SBB         .068         .22268

    2.561     13663360       BV         .614       94.37763

    4.298       126283       VB         .324         .87228

    6.423       104869       BB         .249         .72437

TOTAL AREA=1.4477E+07

MUL FACTOR=1.0000E+00
```

2. **Press [SHIFT] [REPORT] to produce a calibrated report using the method parameters in the active workspace.**

An AREA% (or HEIGHT%) report is generated if a calibrated report cannot be produced.

Choosing a Report Format

The HP 3396 produces AREA% and HEIGHT% reports for uncalibrated analyses and Normalization, External Standard, and Internal Standard reports, based on area or height, for calibrated analyses.

AREA%

This report is the default report produced automatically; no calibration is necessary. Peaks are not named.

1. **Press [LIST] [OP()] [4] to ensure that the Height % report has not been selected.**

Example

```
*  LIST:   OP #  4
REPORT OPTIONS
Suppress local report ..... NO
HEIGHT% report ............. NO   Must be NO to produce AREA% report
Report uncalibrated peaks . NO
Extended report ............ NO
```

2. **Press [AREA%]**

HEIGHT%

This report is similar to the AREA% report but based on height. HEIGHT% reports can be specified in the option 4 or PREP METH dialogs.

1. **Press [OP()] [4] to enter the option 4 dialog.**
2. **Type Y [ENTER] to specify HEIGHT% report. Exit OP() 4 dialog.**
3. **Press [AREA%]**

Example

```
         *  OP #  4
         REPORT OPTIONS
         Suppress local report [Y/N*]: [ENTER]
         HEIGHT% report [Y/N*]: Y [ENTER]
         Replace report title [Y/N*]: [BREAK]

*  AREA%

RUN# 5          OCT 5, 1994 09:01:32

                SAMPLE# 5

SIGNAL FILE: M:SIGNAL.BNC

PEAK FILE : M:Q1EBBE6F.PRO

REPORT #5

HEIGHT%

       RT      HEIGHT     TYPE       WIDTH        HEIGHT%

     .255       32748       BB        .134      .90.91363

     .200       57910     I BH        .134       9.08636

TOTAL HEIGHT= 36021

MUL FACTOR=1.0000E+00
```

NORM (Area or Height)

Calibrated report; corrected responses are used in place of measured responses to compensate for difficulties in detection sensitivity for different components of the sample.

ESTD or ESTD% (Area or Height)

Calibrated report using the external standard calculation based on peak areas or peak heights.

ISTD or ISTD% (Area or Height)

Calibrated report using the internal standard calculation based on peak areas or peak heights.

1. **Prepare a calibration using your choice of calculations.**

Refer to chapter 5 and 6 for detailed information.

2. **Make a run.**

A calibrated report is generated automatically when the calibration criteria are met.

3. **Press [SHIFT] [REPORT] to get additional calibrated reports.**

When you change the calibration file and then reintegrate using ANALYZE, pressing [**SHIFT**] [**REPORT**] will produce a report that reflects the calibration changes.

Adding Extra Information to Reports

Options 4 and 5 and the method and sequence dialogs offer prompts for adding additional information to reports.

Information	How to Include in Report
Sample name	**[PREP]** or **[EDIT] [SEQ]** or OP() 7
Report memo	**[PREP]** or **[EDIT] [SEQ]** or OP() 7
Instrument identifier	**IDENTIFIER** command
Report title	**[PREP]** or **[EDIT] [METH]** or OP() 4
Change AMOUNT label	**[PREP]** or **[EDIT] [METH]** or OP() 4
Extended report	**[PREP]** or **[EDIT] [METH]** or OP() 4
List run parameters	**[PREP]** or **[EDIT] [METH]** or OP() 5
List timetables	**[PREP]** or **[EDIT] [METH]** or OP() 5
List calibration table	**[PREP]** or **[EDIT] [METH]** or OP() 5
List INET set points	**[PREP]** or **[EDIT] [METH]** or OP() 5

Exercising Your Report Options

Options 4 and 5 and the NOTEPAD and IDENTIFIER commands allow you to alter the content of a report. Through the option 4 dialog, you can

- suppress the report produced on the integrator after a real-time run or after reintegration
- select AREA% or HEIGHT% for an uncalibrated report
- specify report title and amount column labeling
- report uncalibrated peaks
- produce an extended calibrated report

1. Press [OP ()] [4] [ENTER] to begin the option 4 dialog.

The HP 3396 prints each dialog prompt and waits for a reply. Selections are shown in brackets with the current choice marked by an *. To retain the selection, press **[ENTER]**. To change it, type in the desired selection and press **[ENTER]**.

The dialog can be ended any time by pressing **[BREAK]**. Everything entered before the **[BREAK]** will be retained. Entries that were not reached will have the values they had before the dialog started.

Example

```
* OP # 4

REPORT OPTIONS
Suppress local report [Y/N*]:
```

Y: The integrator does not print a report at the end of a real-time run or after reintegration on its printer/plotter. Printing a report on an external device is not affected by this response.

N: The unit produces a report after a real-time run or after reintegration.

`HEIGHT% report [Y/N*]:`

Y: Selects HEIGHT% as the uncalibrated report type. Selecting HEIGHT% bases the calculation for the uncalibrated report on height. For calibrated reports, the choice of area or height as the basis for calibration response factors is made in the **[PREP] [CALIB]** dialog when the Calibration Table is created. See chapter 5.

N: Selects AREA% as the uncalibrated report type.

`Replace report title [Y/N*]:`

Y: Allows you to enter a title for your report. The title can be up to 42 upper or lowercase characters in length, including spaces and punctuation. To erase an existing report title, enter the option 4 dialog, specify **Y** to this prompt, and then press **[ENTER]** to the `Report title:` prompt.

N: No report title replacement. The current title (if there is one) remains.

`Replace amount label [Y/N*]:`

Y: You will be prompted for an amount label. The amount label can be up to 10 upper- or lowercase characters in length, including spaces and punctuation. The label you enter will be used instead of AMOUNT as the column heading for the results column in the report for a calibrated procedure. Replace AMOUNT with concentration or measurement units.

To erase your amount label and have the column labeled AMOUNT again, enter **Y** to this prompt and press **[ENTER]** when asked for the amount label.

N: AMOUNT retained as column heading.

```
Report uncalibrated peaks [Y/N*]:
```

Y: Calibrated reports include uncalibrated *and* calibrated peaks. If the response factor for uncalibrated peaks is zero, the reported AMOUNT will be zero (see chapter 5). A response factor for all uncalibrated peaks is set during calibration or by using option 3. This can be helpful when diagnosing problems with calibration calculations.

N: Calibrated reports contain only calibrated peaks.

```
Extended report [Y/N*]:
```

Y: The integrator produces an extended version of a calibrated report including computed peak types, areas, width, heights, CAL#s, amounts, names, and group information (if present). The columns of analysis information shown in an extended report are

```
RT TYPE   AREA   WIDTH   HEIGHT   CAL#   AMOUNT    NAME
```

N: The integrator produces a standard calibrated report consisting of the following column headings

```
RT       AREA TYPE   CAL#     AMOUNT
```

Peak grouping information (if present) is also included.

Press [OP ()] [−] [4] to reset current option 4 selections back to the default selections.

2. **Press [LIST][OP ()] [4] to list the current option 4 selections.**

Option 5: Listing Additional Information with Your Report

Besides allowing you to store reports, option 5 allows you to

- choose the font size
- produce a report on an external printer
- list the run parameters after the report
- list the timetable after the report
- list the Calibration table after the report
- list the remote method after the report

These listings occur only after a run or reintegration. They are not printed with the report when the **[AREA%]** or **[REPORT]** keys are used. Begin the option 5 dialog by pressing

1. **Press [OP ()] [5] [ENTER] to begin the option 5 dialog.**

As in option 4, press **[ENTER]** to retain current selections. Abort the dialog at any point by pressing **[BREAK]**.

After questions about report storage, option 5 continues.

Example

```
* OP # 5
PRINT & POST-RUN LIST OPTIONS
Large font [Y*/N]: [ENTER]
Store post-run report [Y/N*]: Y [ENTER]
Device [M*]: [ENTER] or name of external storage device
External post-run report [Y/N*]:
```

Y: The unit prints the report on an external device specified in response to the “Printer address” prompt which follows.

```
Printer Address [0 ]:
```

Enter the address of the external printer to receive the report. Use the **SYSTEM** command (**[S] [Y] [ENTER]**) to find the address of the external HP-IL printer. Numbers from 8 to 30 are valid. HP-IB printers (addresses 1−7) are accessed with the **XADDRESS** command.

N: The integrator does not send the report to an external printer.

```
List run parameters [Y/N*]:
```

Y: The integrator prints the run parameters used for the analysis after the report.

N: The run parameters are not printed after the report.

```
List timetable [Y/N*]:
```

Y: The HP 3396 lists Timetable Events after the report. If no Timetable exists, the integrator prints `EMPTY`.

N: The Timetable is not printed after the report.

```
List calibration table [Y/*N]:
```

Y: The integrator lists the Calibration Table after the report.

N: The Calibration Table is not printed after the report.

```
List remote method [Y/*N]:
```

Y: The integrator lists the INET instrument set points associated with the analysis after the report.

N: INET instrument set points are not listed.

```
Form-feed before report [Y/N*]:
```

Y: The integrator does a form feed before starting to print the report.

N: The report starts immediately after the chromatogram.

`Form-feed after report [Y/N*]:`

Y: The integrator does a form feed after the end of each report.

N: No form feed is implemented after reports.

`Skip perforations in report [Y/N*]:`

Y: The integrator does not print over page breaks in a report when the Top of Form has been set accurately. At this point, the integrator will ask

`Skip perforations in plot [Y/N*]:`

Y: Plotting is suspended over page breaks when the top of form has been set accurately.

N: Plotting continues over page breaks.

N: The integrator prints over page breaks when they occur during a report.

Press [OP ()] [−] [5] to reset current option 5 selections back to the default selections.

2. **Press [LIST] [OP ()] [5] to list the current option 5 selections.**

NOTEPAD Command

Use **NOTEPAD** to make notes, such as chromographic conditions, analysis data, or the like, on the printer/plotter.

1. **Type NOTEPAD [ENTER] then enter the desired text directly on the report.**

Example

```
* NOTEPAD @
(USE BREAK OR CONTROL-Y TO END)
Testing column conditioning;
First pass-operator is Kate
*
```

2. **Terminate the text entry by pressing [BREAK].**

The unit will return to system command mode without printing the word BREAK. This notation cannot be stored with the method, report, or in the active workspace.

. IDENTIFIER Command

Use **IDENTIFIER** to identify the data source at the top of the report format.

1. **Type [I] [D] [space] then enter the desired text (up to 12 alphanumeric characters) and press [ENTER].**

Once an IDENTIFIER is set, it will appear on every report until it is changed or deleted.

Example

```
* ID Flavors Lab1
* REPORT
RUN#   19    FEB 18, 1995   09:24:00
IDENTIFIER : Flavors Lab1
      the report continues ...
```

2. **Type [I] [D] [“] [”] to delete an identifier.**

Storing and Reprinting Reports

Reports may be saved through option 5 and reprinted using a BASIC program or the HP PEAK-96 Information Manager.

1. **Press [OP ()] [5] [ENTER] to begin the option 5 dialog.**

```
Store post-run report [Y/N*]:
```

2. **Type Y [ENTER] to store a report.**

The integrator stores the report in LIF ASCII format, in a report file on the device specified below.

If you answer **N** and a report is not stored in a file, the report can be reproduced locally by pressing **[REPORT]** until the current peak data in the active workspace are overwritten by new data or erased.

```
Device [M*]:
```

3. **Enter a disk drive name for the report file.**

The default disk is M:, the internal memory disk. Be sure that any external disk drive indicated is fully operational before beginning the run. An error message will not be printed until *after* the run if the disk drive is not operational. Use H: to send reports to HP Peak-96 Information Manager. See chapter 7 for a complete list of valid disk drive names.

4. **Reprint a stored report using the HP Peak-96 Information Manager or using the BASIC program listed below.**

Programs can be created only when BASIC is installed.

```
110     ! This program lists a disk file to the printer.
120     ! When entering the filename, you must specify a disk drive.
130     !
140     DIM A$(132)
```

```
150     PRINT ‘‘ENTER FILE [d:x.y]’’;
155     ! d: represents the disk drive, e.g. M:, A:, B:
157     ! x represents the 8-character filename
158     ! y represents a 3-character file extension type, e.g. .RPT
160     INPUT A$
170     OPEN #3: NAME A$
180     WHEN EXCEPTION IN
190     DO
200     READ #3:A$
210     PRINT A$
220     LOOP
230     USE
240     IF EXTYPE=7105 THEN ! Normal end of file.
250     PRINT
260     PRINT ‘‘END OF FILE’’
270     PRINT
280     ELSE
290     PRINT EXTEXT$(EXTYPE)
300     END IF
310     END WHEN
320     END
```

Understanding Reports

The report has three sections. Identification information leads the report, followed by columns of data from the analysis and additional calculation information.

Identification Information

The report heading contains the current date and time and information identifying the run and the sample. The first line of the report always contains the run number and the integrator date and time.

The next lines in the report heading may be optionally inserted before the calculation procedure.

Report Identification Information

`RUN# 7 JAN 15, 1995 21:22:08`	set clock for correct DATE and TIME
`SAMPLE NAME: SAMPLE NAME`	
`REPORT MEMO`	
`IDENTIFIER: LAB1-GC3`	identifier indicates analysis instrument
`SIGNAL FILE: M:SIGNAL.RAW`	filename assigned by integrator
`REPORT FILE: M:Q1FE0F80.RPT`	filename assigned by integrator
`REPORT TITLE`	
`ISTD PEAK NOT MATCHED`	error messages
`SAMPLE# 90`	valid sample number provided by INET or via the SAMPLE # cable on the HP 3396 rear panel

Analysis Information

The body of the report starts with the name of the calculation procedure used to generate the report. Calibrated reports indicate whether the calculation is based on AREA or HEIGHT; for example, ISTD—HEIGHT is a calibrated report using the ISTD calculation based on peak heights.

For a complete list of calibrated reports see *Choosing Your Report Format* at the beginning of this chapter. A calibrated report is illustrated below. The columns of information that may be found in either a calibrated or uncalibrated report are listed below.

RT The retention times of the peaks in the run, in order of increasing retention time. Retention time is the unique identifier for a peak.

AREA The area in counts for each peak. One count is 1/8 μV-sec. The area given is the final area after baseline determination and/or correction have been done.

HEIGHT The height in counts for each peak. One count is 1/8 μV. The height given is the final height after baseline determination and correction have been done.

TYPE Up to 4 characters which indicate how the peak detection and quantitation processed the peak. The TYPE provides information about baseline construction. The table on the next page lists all the HP 3396 peak-type codes.

HP 3396 Codes for Peak Types

WARNING Codes (**>**, **I**, **<**, **N**)
Only one warning code can be printed per peak. If a peak is eligible for more than one, the highest priority code is printed. The codes are listed in priority order.

> (Over-range)	The peak exceeds 1000 mV (analog input). Reduce the signal level at the chromatograph or reduce the sample size to allow quantitation.
I (Incomplete)	The peak ends prematurely, after its apex but before the signal returned to baseline, valley, or tangent points. Perhaps STOP occurred or there was a forced return to baseline caused by an INTG ( ) function.
< (Under-range)	The (analog) signal was less than −10 mV. Adjust the chromatograph's detector output for electrical zero.
N (Negative peak)	A negative peak was inverted by integration function INTG #11 and then processed as a normal (positive) peak.

SOLVENT Codes (**S**, **T**)
Solvent codes indicate a peak is treated in a special manner with respect to baseline construction.

S (Solvent)	The peak is designated a solvent peak.
T (Tangent Skimmed)	The peak is tangent-skimmed from the downslope of a peak declared to be a solvent.

BASELINE Codes (**B**, **V**, **P**, **H**)
Baseline codes indicate how a peak starts and ends and how the chromatographic baseline is constructed.

B (Baseline)	Peak begins or ends on baseline.
V (Valley point)	A valley point occurs when a peak begins before the previous peak ends by returning to baseline.
P (Baseline penetration)	Baseline is penetrated, then reset to the lowest point at the beginning or end of the peak.
H (Horizontal baseline)	Baseline is horizontal and extends from the last declared baseline point.

TOTAL AREA (or HEIGHT) is the sum of the areas (or heights) of all of the peaks integrated.

MUL FACTOR is a number used to multiply the final calculated value for unit conversions or to correct for changes in sample dilution. More details about `MUL FACTOR` can be found in chapter 5.

Uncalibrated reports also list `WIDTH` and `AREA%` or `HEIGHT%` columns.

WIDTH During the run, the integrator computes the width of every peak in the run and reports the results. Width is computed in units of decimal minutes using the determined height and area. Use values listed under `WIDTH` to help you select the proper value for peak width.

$$\text{Width} = \text{Area}/(\text{Height} \times 60)$$

AREA% The area of a peak as a percentage of the total area accumulated during the run.

HEIGHT% The height of a peak as a percentage of the total height accumulated during the run.

Sample Calibrated Report

```
RUN#    65          JUL  5, 1995  09:01:32

                         SAMPLE#    7

SIGNAL FILE: M:SIGNAL.BNC

TECH PROPIONIC ACID

ESTD-AREA
    RT     AREA   TYPE  CAL#   AMOUNT
 2.561 13663360    BV    .1  100.714
 4.298   126283    VB    .2    1.042
 6.423   104869    BB   .3R    1.052

TOTAL AREA=1.4477E+07
MUL FACTOR=1.0000E+00
```

The calibrated report contains information that does not appear in the uncalibrated report.

CAL # The number of the peak in the Calibration Table. **R** after the peak indicates a reference peak. **&** after a peak indicates a reference and ISTD peak. Nonreference ISTD peaks are indicated by an **S** in the report.

AMOUNT The result calculated by the method. You may change the labeling of this column to reflect the appropriate measurement units or percent concentration.

ISTD AMT The internal standard amount indicated in the Calibration Table and in option 7.

SAMPLE AMT The amount of sample (greater than 0) for an ISTD% or ESTD% calculation.

The last three columns of information in the calibrated report have to do with peak groupings. Group data are present only if

- peak grouping by calibration number is defined and enabled in the Calibration Table and
- any of the peaks in a group are found.

GRP# The number of the peak grouping listed in the Calibration Table.

AMOUNT The combined amounts of the peaks listed under a group in the Calibration Table.

NAME The name for a group of peaks indicated in the Calibration Table.

Standard vs Extended Report

```
RUN#    65          JUL  5, 1995  09:01:32

                         SAMPLE#    7

SIGNAL FILE: M:SIGNAL.BNC

TECH PROPIONIC ACID

ESTD-AREA
    RT     AREA  TYPE  CAL#   AMOUNT
 2.561 13663360    BV    .1  100.714
 4.298   126283    VB    .2    1.042
 6.423   104869    BB   .3R    1.052

TOTAL AREA=1.4477E+07
MUL FACTOR=1.0000E+00
```

```
RUN#    65          JUL  5, 1995  09:01:32

                         SAMPLE#    7

SIGNAL FILE: M:SIGNAL.BNC

TECH PROPIONIC ACID

ESTD-AREA
    RT TYPE     AREA WIDTH HEIGHT CAL#   AMOUNT   NAME
  .138   PP     5076  .015   5749           .000
  .200   PB    57910  .021  45993           .000
  .295   PB   487595  .022 364146           .000
 2.220  SBB    32238  .068   7956           .000
 2.561   BV 13663360  .614 371133    1   100.714   PPA
 4.298   VB   126283  .324   6495    2     1.042   IBTA
 6.423   BB   104869  .249   7032   3R     1.052   BTA

TOTAL AREA=1.4477E+07
MUL FACTOR=1.0000E+04
```

Extended reports add columns for WIDTH and peak NAMES to the calibrated report and include both AREA and HEIGHT counts. (When using the larger font, *either* AREA *or* HEIGHT is included in the extended report.)

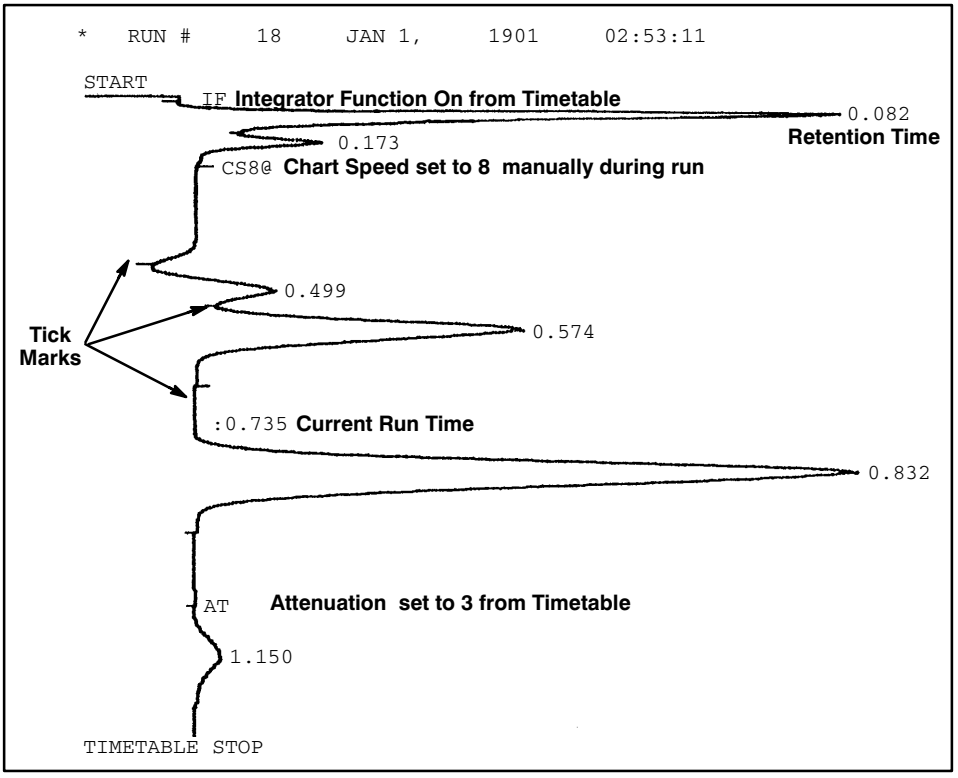
NAME The peak name entered in the Calibration Table. See chapter 5.

Interpreting Baseline Construction and Area Allocation

The chromatogram and the report contain information indicating baseline construction and peak area allocation for the run. The chromatogram shows peak retention times; one-, two-, and four-letter mnemonics for executed functions; the position of the baseline at the start and stop of the run, and tick marks (optional). The peak type codes in the report and the allocation of area among the peaks also give clues to construction.

The information in this section is intended as a guide for reading your chromatogram and its accompanying report.

HP 3396 Chromatogram



Tick Marks

Tick marks are the upstrokes and downstrokes on the chromatogram that separate peaks. A downstroke marks the recognition of the beginning of a peak, and an upstroke marks the recognition of the end. If peaks are too close together, the upstroke may not be printed. The downstroke marking the beginning of the following peak also marks the end of the preceding peak.

Large tick marks (3 mm) locate baseline points. The marked peak began and/or ended on baseline. Small tick marks (1.5 mm) indicate unresolved baseline. Valley points between merged peaks, the starts and ends of tangent-skimmed peaks, or a penetrated baseline are indicated with short tick marks. See chapter 3 for instructions about turning on tick marks.

Key Codes and Mnemonics

Various letters and symbols are printed on the plot during a run in integration mode to indicate that some function was executed during the run.

Any Plot Control function executed during a run is echoed on the plot by a two-letter code. These codes are listed in Chapter 2. The **[START]** key is echoed on the chart at the beginning of the run. The **[STOP]** key is echoed if it is entered manually, and `TIMETABLE STOP` is printed if a STOP is executed from the Timetable.

If the **[TIME]** key is pressed during a run, the current run time is printed preceded by a colon to distinguish it from a retention time. See above.

Retention Time

A peak retention time (printed at the apex of the peak and in the report) is the time between the start of the run and the apex. Retention times are not printed for peaks that do not meet threshold or peak width criteria.

Missing Peaks

When a retention time appears on the plot but not in the report, the peak was recognized and integrated but did not meet the minimum area requirement or peak width criteria. When area reject **[AR REJ]** is set above the calculated area for a peak, the peak will be rejected and not appear in the report. Similarly, when the peak width **[PK WD]** is set for more than four times the actual width of a peak, the peak may not appear in the report. See chapter 3 for more information about area reject and peak width.

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